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Discovery of *trans*-4-[1-[[2,5-Dichloro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid: An Orally Active, Selective Very Late Antigen-4 Antagonist

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We have focused on optimization of the inadequate pharmacokinetic profile of *trans*-4-substituted cyclohexanecarboxylic acid **5**, which is commonly observed in many small molecule very late antigen-4 (VLA-4) antagonists. We modified the lipophilic moiety in **5** and found that reducing the polar surface area of this moiety results in improvement of the PK profile. Consequently, our efforts have led to the discovery of *trans*-4-[1-[[2,5-dichloro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (**14e**) with potent activity (IC_{50} = 5.4 nM) and significantly improved bioavailability in rats, dogs, and monkeys (100%, 91%, 68%), which demonstrated excellent oral efficacy in murine and guinea pig models of asthma. Based on its overall profile, compound **14e** was progressed into clinical trails. In a single ascending-dose phase I clinical study, compound **14e** exhibited favorable oral exposure as expected and had no serious adverse events.

Introduction

The pathogenesis of inflammatory and autoimmune diseases such as asthma,[1] rheumatoid arthritis,[2] type 1 diabetes mellitus,[3] psoriasis,[4] multiple sclerosis (MS),[5] inflammatory bowel disease,[6] and hepatitis C[7] is commonly characterized by an influx of activated leukocytes to affected tissues. The sustained accumulation of inflammatory cells keeps chronic inflammatory conditions in the tissue, resulting in tissue damage and dysfunction. It is well-known that the integrin very late antigen-4 (VLA-4, $\alpha_4\beta_1$, CD49d/CD29)[a] is intrinsically involved in the development of this pathogenesis. VLA-4 is a heterodimeric glycoprotein receptor consisting of α_4 and β_1 chains that is constitutively expressed on the surface of almost all leukocytes.[8] It binds vascular cell adhesion molecule-1 (VCAM-1, CD106) expressed on cytokine-stimulated endothelial cells and the alternatively spliced portion of the type III connecting segment of fibronectin (FN)[9,10] and mediates the process of adhesion, migration, and activation of inflammatory cells at the site of inflammation. Therefore, a blockade of the interaction of VLA-4 with the ligands would alleviate the inappropriate cellular process and would be useful in the treatment of inflammatory and autoimmune diseases. To date, it has been reported that anti-α_4 antibodies and small-molecule VLA-4 antagonists demonstrate inhibition of leukocyte infiltration into extravascular tissue and prevent tissue damage in a wide variety of inflammatory animal models.[11] Furthermore, clinical trials in phase III using a humanized monoclonal anti-α_4 antibody, natalizumab, for the treatment of MS and Crohn's disease provided excellent results and strongly validated the proof of concept of this target as a therapeutic agent. Consequently, natalizumab has been approved by the FDA for the treatment of both MS and Crohn's disease.[12] On the other hand, oral small-molecule VLA-4 antagonists possessing similar efficacy and an appropriate half-life in comparison with natalizumab are still considered to be beneficial from a safety and cost point of view. Over the past decade or more, intensive efforts to discover and develop new candidate small molecule VLA-4 antagonists have been seen in the pharmaceutical industry. However, there are currently only a few candidates in clinical trials for the treatment of MS and Crohn's disease, and none of these compounds has yet reached the marketplace.

The small-molecule VLA-4 antagonists that have so far been progressed into clinical trials are classified into two major structures: (1) LDV mimics, whose sequence is responsible for interaction with FN, such as the highly selective VLA-4 antagonist Bio-1211 (Figure 1)[13] or (2) *N*-acylphenylalanine-based compounds[14] such as valategrast (R411, Figure 1), many of which exhibit dual inhibitory activity for integrin $\alpha_4\beta_1$, as well as $\alpha_4\beta_7$.

For several years, our efforts have been directed toward the development of orally active, potent, and selective VLA-4 antagonists based on LDV-derived compound **3** as the initial lead (Figure 2),[15] which was identified through a hit to lead research program. The key issue was to address the poor pharmacokinetic (PK) profile, including the extremely high

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[a]Abbreviations: VLA-4, very late antigen-4; CD, cluster of differentiation; VCAM-1, vascular cell adhesion molecule-1; PSA, polar surface area; HBD, hydrogen bond donors; HSA, human serum albumin; BALF, bronchoalveolar lavage fluid; BHR, bronchial hyper-responsiveness; LDV, leucine-aspartate-valine.

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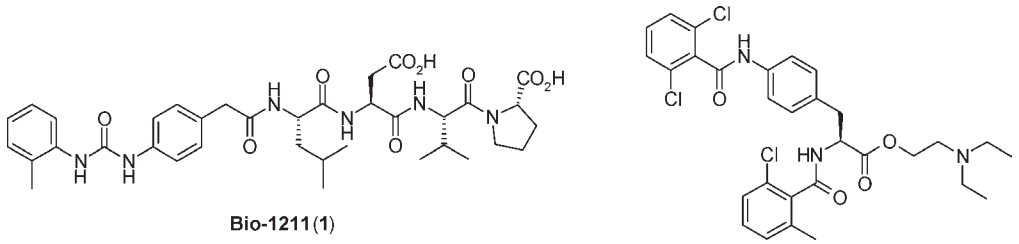


Figure 1

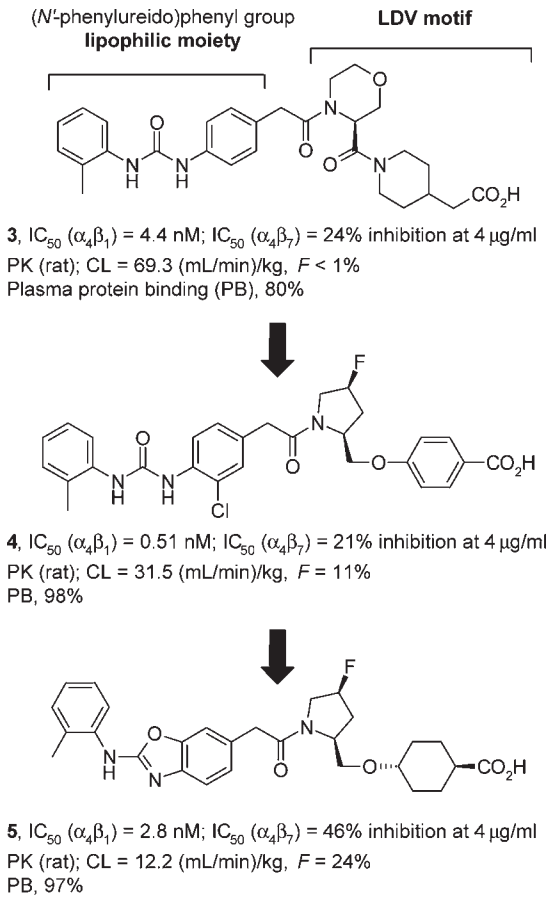


Figure 2

plasma clearance (CL = 69.3 (mL/min)/kg) and low oral bioavailability ($F < 1\%$) in rats, which have commonly been observed in most VLA-4 antagonists, likely because of their high biliary excretion[16] rate due to a multidrug resistance-associated protein 2 (MRP2) mediated process,[17] while retaining the VLA-4 inhibitory activity. Thus, to establish a pertinent structure−PK profile and structure−activity relationships, we have worked on modification of both the LDV motif and the (*N*′-phenylureido)phenyl group in **3**. In the course of this study, it was revealed that benzoic acid derivative **4** with the chlorine atom at the 3-position of the central benzene in the (*N*′-phenylureido)phenyl group and cyclohexanecarboxylic acid derivative **5** with 2-(2-methylphenylamino)benzoxazolyl group instead of (*N*′-phenylureido)phenyl group showed a somewhat improved PK profile in part due to increased plasma protein binding (Figure 2).[18] In addition, the difference in the clearance between compounds **4** and **5** seemed to correlate to the polar surface area (PSA) value of the lipophilic moiety in each compound (36.6 Å^2 for **4**; 30.8 Å^2 for **5**), the number of hydrogen bond donors (HBD, 2 for **4**; 1 for **5**), or both.[19]

From these results, we considered that structural modification of the lipophilic moiety to reduce PSA and HBD could lead to further improvement of the PK profile. It might be presumed that such a modification could enhance the affinity for certain plasma proteins such as albumin and make it easy for the modified compounds to employ the plasma proteins as carriers in the blood. Although the enhancement of plasma protein binding generally results in a drop of activity *in vivo*, with acceptable activity in the presence of plasma and with sufficient concentrations, we believed that the compounds could exert *in vivo* efficacy. Thus, as illustrated in Figure 3, we focused our efforts on incorporation of the urea in the (*N*′-phenylureido)phenyl group into various cyclic structures leading to 2-(arylamino)benzoxazolyl (I), 4-(2-benzoxazolylamino)phenyl (II), and 4-(hetroarylcarboxamido)phenyl (III) groups while fixing the *trans*-4-[(*4S*)-fluoro-(*2S*)-pyrrolidinylmethyloxy]cyclohexanecarboxylic acid scaffold in **5**, which was highly optimized as a novel LDV motif.[18b]

We herein report on optimization of the lipophilic moiety in **5**, which led to the identification of a series of 1-methyl-3-indolyl derivatives with a significantly improved PK profile in rodents, and the subsequent fine-tuning of the substituents in the 4-(1-methyl-3-indolylcarboxamido)phenyl group and the pyrrolidine ring as well as the selection of compound **14e** as a clinical candidate.

Chemistry

The target compounds were synthesized according to the general procedure outlined in Scheme 1. Thus, arylacetic acids **7**−**9** were condensed with pyrrolidine **10** using EDC and HOBt. The resulting amides were subjected to basic hydrolysis to afford *trans*-4-substituted cyclohexanecarboxylic acids **11**−**14**. The syntheses of the intermediates **7**−**10** in Scheme 1 are fully presented in Schemes 2−5.

The preparation of (6-benzoxazolyl)acetic acids **7a**−**f** is depicted in Scheme 2. Condensation of methyl (4-amino-3-hydroxyphenyl)acetate (**15**)[20] with *o*-tolylacetic acid was carried out using triphenylphosphine/hexachloroethane,[21] followed by basic hydrolysis to give the [2-(2-methylbenzyl)-6-benzoxalolyl]acetic acid (**7a**). After protection of the hydroxyl group in 2,3-difluoro-6-nitrophenol (**16**) with a benzyl group, the benzyl ether was treated with dimethyl malonate in the presence of NaH to give **17**, which was converted to the methyl ester **18** by basic hydrolysis and decarboxylation followed by esterification. Hydrogenation of **18** over Pd/C resulted in reduction of the nitro group and deprotection of the benzyl group to give the aminophenol **19**. Cyclization of both **15** and **19** to the corresponding benzoxazole derivatives was achieved

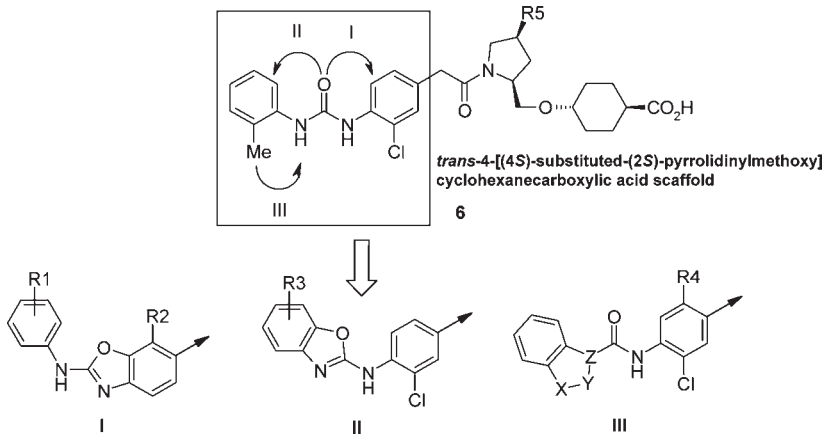
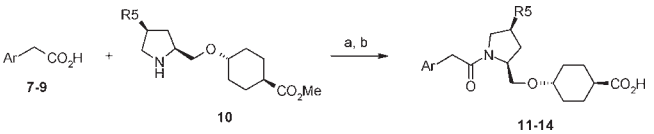


Figure 3

Scheme 1[a]



[a] Reagents and conditions: (a) EDC·HCl, HOBt, Et_3N, DMF; (b) aq NaOH, THF.

by condensation with commercially avalilable isothiocyanates or 3-fluoro-2-methylaniline/thiocarbonyl diimidazole and subsequent treatment with HgO.[20] The benzoxazoles were hydrolyzed under a basic condition to provide (2-arylamino-6-benzoxazolyl)acetic acids **7b**−**f**.

[4-(2-Benzoxazolyl)aminophenyl]acetic acids **8a**−**e** were prepared as shown in Scheme 3. Nucleophilic displacement of 2-chlorobenzoxazole with ethyl (4-amino-3-chlorophenyl)acetate (**20a**)[22] and subsequent basic hydrolysis gave [4-(2-benzoxazolyl)amino-3-chlorophenyl]acetic acid (**8a**). Methyl (4-amino-3-chlorophenyl)acetate (**20b**)[23] was treated with thiophosgene in the presence of $CaCO_3$ to afford isothiocyanate **22**,[24] which was subjected to the cyclization procedure described in Scheme 2 with aminophenols **23a**−**d** to afford [4-(2-benzoxazolyl)aminophenyl]acetic acids **8b**−**e**.

[4-(Heteroarylcarboxamido)phenyl]acetic acids **9a**−**n** were prepared as shown in Scheme 4. Indoline (**24**) was converted to **9a**,**b** via urea formation with anilines **20a** or **20c** using triphosgene followed by basic hydrolysis. Following treatment of 1-alkylindole-3-carboxylic acid **27c**−**e** with thionyl chloride, condensation of the resultant acid chlorides with anilines **20**, and basic hydrolysis afforded **9e**−**i**. 1-(4-Methoxybenzyl)indazole-3-carboxylic acid (**27b**) was also converted to acid chloride, which was condensed with aniline **20c**, followed by deprotection of 4-methoxybenzyl group by TFA treatment and basic hydrolysis to afford **9d**. Alternatively, in the case of **9c** and **9j**−**n**, an amide bond forming reaction of **20** with 1-indole-3-carboxylic acid (**27a**), benzisothiazole-3-carboxylic acid (**27f**), benzisoxazole-3-carboxylic acid (**27g**), and isoquinoline-1-carboxylic acid (**27h**) was performed by using EDC and HOBt.

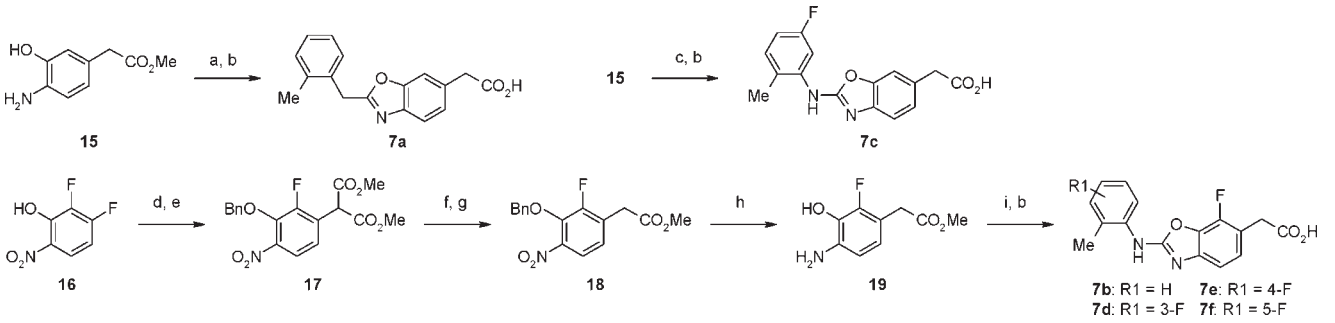
We next turned our attention to the preparation of methyl *trans*-4-substituted cyclohexanecarboxylates **10a**−**d** starting with the methyl benzoates **28a**−**c** (Scheme 5).[25] Thus, after deprotection of the *tert*-butoxycarbonyl (Boc) group of **28a**−**c**, reduction of the benzene ring in **28a**−**c** was achieved by hydrogenation (10 atm) using Rh/Al_2O_3 as the catalyst to afford a mixture of the corresponding *cis*- and *trans*-4-substituted cyclohexane derivatives in about a 6:1 ratio, which without separation were reprotected with a Boc group to furnish **29a**−**c**. In the case of **29c**, the hydroxyl group was protected with a benzyloxymethyl (BOM) group to give **29d**. Treatment of the *cis*-rich isomers **29a**,**b** and **29d** with sodium methoxide in refluxing methanol resulted in a mixture of the *cis*- and *trans*-isomers in a ratio of 1:1. Following esterification with (trimethylsilyl)diazomethane, isolation of the *trans*-isomers was successfully performed by flash column chromatography to afford the *trans*-isomers **30a**−**c**. Conversion of the BOMO group in **30c** to the substituents of **30d**−**f** was carried out accoding to the procedure previously reported by us.[25] Removal of the Boc function of **30a**,**b** and **30e**,**f** by TFA treatment produced the methyl *trans*-4-[(4*S*)-substituted-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylates **10a**−**d**.

Results and Discussion

Optimization of Lipophilic Moiety. The *trans*-4-substituted cyclohexanecarboxylic acid derivatives **11**−**14** were initially screened 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 with or without the addition of 3% human serum albumin (HSA). In addition, regarding compounds showing potent activity with IC_{50}'s < 10 nM in the binding assay, we measured the level of VLA-4 inhibition in the serum collected at 15 min post-oral dosing at 10 mg/kg in mice. By comparison with a calibration curve, the compound concentration in serum was estimated. At this point, because it was found that the T_{max} of this series of compounds was within nearly 30 min in the pharmacokinetic study that we previously reported,[18a] we set the collection time of the blood samples to 15 min.

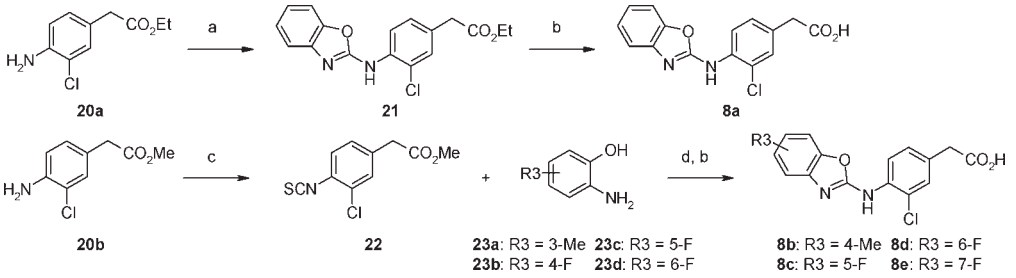
The evaluation results of 2-(benzyl or phenylamino)benzoxazole derivatives are summarized in Table 1. First, to investigate how the NH at the 2-position in the benzoxazole unit in **5** affects the activity and oral exposure in mice, we replaced the NH with CH_2. As a result, it was found that this modification caused significant loss of potency (**11a**, 33% inhibition at 2 μM). On the other hand, we reported that the introduction of a fluorine atom into the 5-position in the terminal benzene in compound **4** led to somewhat of an improvement of the pharmacokinetic properties while retaining the activity.[22] Therefore, we introduced a fluorine atom to the 5-position in the terminal benzene in **5** as an R1

Scheme 2[a]



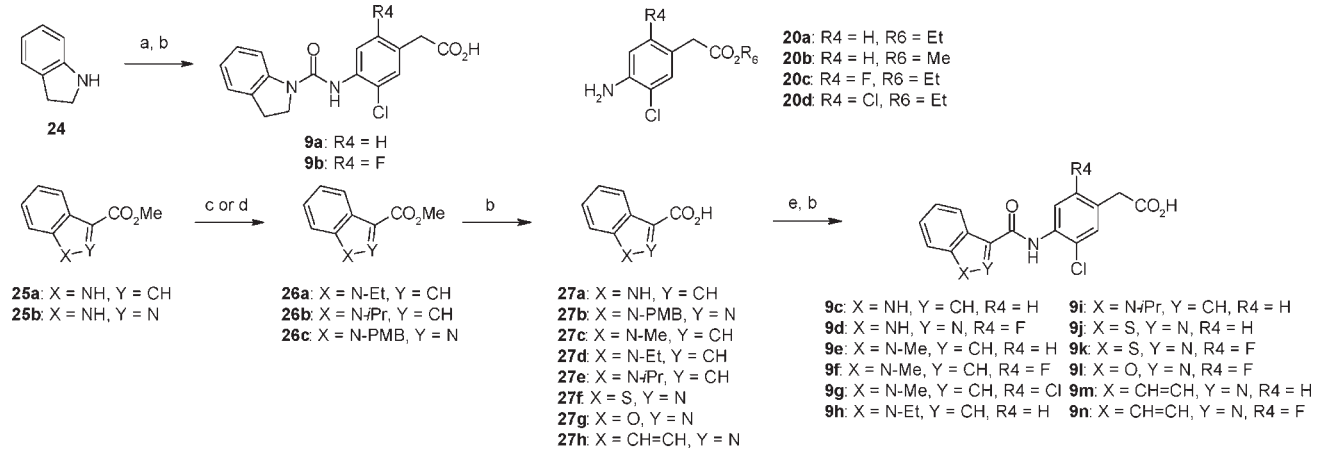
[a] Reagents and conditions: (a) *o*-tolylacetic acid, hexachloroethane, Ph_3P, Et_3N, MeCN; (b) aq NaOH, THF/MeOH; (c) 5-fluoro-2-methylphenyl isothiocyanate, MeOH, then HgO, MeOH, reflux; (d) benzyl bromide, K_2CO_3, DMF; (e) dimethyl malonate, NaH, NMP; (f) 2 N NaOH, MeOH, reflux, then conc. HCl; (g) conc. H_2SO_4, MeOH, reflux; (h) H_2, 5% Pd/C, MeOH; (i) (i) *o*-tolyl isothiocyanate or 4- or 5-fluoro-2-methylphenyl isothiocyanate, MeOH, then HgO, MeOH, reflux (for **7b**, **7e**, and **7f**), (ii) 3-fluoro-2-methylaniline, thiocarbonyl diimidazole, THF then HgO, THF (for **7d**).

Scheme 3[a]



[a] Reagents and conditions: (a) 2-chlorobenzoxazole, xylene, reflux; (b) aq NaOH, THF; (c) thiophosgene, $CaCO_3$, $H_2O/CHCl_3$; (d) HgO, toluene, reflux.

Scheme 4[a]

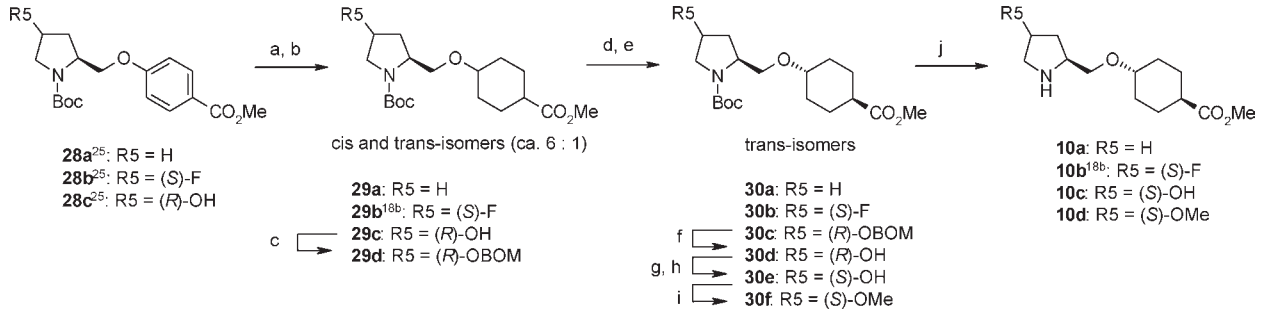


[a] Reagents and conditions: (a) anilines **20**, triphosgene, pyridine, CH_2Cl_2; (b) aq NaOH, THF; (c) ethyl or isopropyl iodide, NaH, DMF (for **26a**,**b**); (d) K_2CO_3, PMBCl, DMF (for **26c**); (e) (i) $(COCl)_2$, CH_2Cl_2, then anilines **20**, Et_3N, CH_2Cl_2, reflux, (ii) TFA, anisole, reflux (only for **9d**), or EDC·HCl, HOBt, Et_3N, DMF, anilines **20**.

substituent. In addition, we also introduced a fluorine atom to the 7-position in the benzoxazole ring. Although the introductions were relatively tolerated in activity without and with 3% HSA compared with **5**, no enhancement of the estimated serum concentration was observed. Next, we carried out introduction of a fluorine atom to the 3-, 4-, or 5-position to R1 while fixing R2 = F. Only 5-F-benzoxazole **11f** retained activity (IC_{50} = 2.8 nM), showing 10 times less potent activity in the presence of 3% HSA (IC_{50} = 439 nM) compared with **5**, which was likely due to an increase of protein binding. Additionally, it was found that the estimated serum concentration was 2.4 times higher than that of **5**.

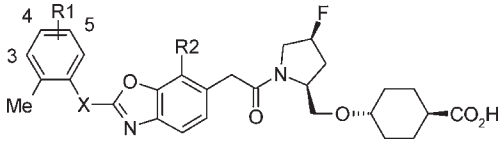
Regarding compounds that had the terminal phenylaminocarbonyl group in compound **4** transformed into the corresponding benzoxazole ring, we investigated the effect of substituents (none, 4-Me, 5-F, 6-F, and 7-F) on the benzoxazole ring on the activity and estimated serum concentration (Table 2). In this modification, we fixed a chlorine atom at the 3-position on the central benzene ring based on the positive effect on the pharmacokinetic properties in rodents and dogs that we previously reported.[18a] Among them, nonsubstituted benzoxazole **12a** and 5-F-benzoxazole **12c** retained the activity (**12a**, IC_{50} = 5.9 nM; **12c**, IC_{50} = 7.3 nM), including in the presence of 3% HSA (**12a**, IC_{50} = 138 nM; **12c**, IC_{50} = 223 nM).

Scheme 5[a]



[a] Reagents and conditions: (a) TFA, CH_2Cl_2, then 5% Rh/Al_2O_3, H_2 (10 atm); (b) Boc_2O, Et_3N, $MeCN/H_2O$; (c) BOMCl, iPr_2EtN, CH_2Cl_2; (d) NaOMe, MeOH, reflux; (e) $TMSCHN_2$, benzene/MeOH then separation by flash column chromatograpy; (f) H_2, 5% Pd/C, MeOH; (g) formic acid, DIAD, Ph_3P, THF; (h) $NaHCO_3$, THF, H_2O; (i) MeI, NaH, DMF; (j) TFA, CH_2Cl_2.

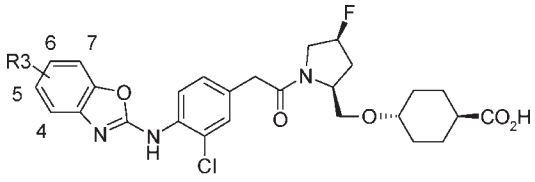
Table 1. Inhibitory Activity and Estimated Serum Concentration of 2-(Benzyl or Phenylamino)benzoxazole Derivatives **5** and **11a−f**



5 and **11a-f**

compd	R1	R2	X	IC_{50} (nM) (−/+ 3% HSA)	estimated serum concentration (ng/mL)
5	H	H	NH	2.8/45	373
11a	H	H	CH_2	33% inhibition at 2 μM/7% inhibition at 2 μM	Not tested
11b	H	F	NH	3.0/70	363
11c	5-F	H	NH	4.4/131	448
11d	3-F	F	NH	47/13% inhibition at 1.8 μM	Not tested
11e	4-F	F	NH	12/1680	Not tested
11f	5-F	F	NH	2.8/439	912

Table 2. Inhibitory Activity and Estimated Serum Concentration of 4-(2-Benzoxazolylamino)phenyl derivatives **12a−e**



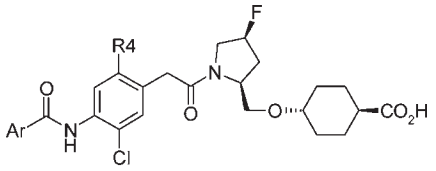
12a-e

compd	R3	IC_{50} (nM) (−/+ 3% HSA)	estimated serum concentration (ng/mL)
12a	H	5.9/138	508
12b	4-Me	12/290	Not tested
12c	5-F	7.3/223	358
12d	6-F	20/630	Not tested
12e	7-F	40/1032	Not tested

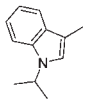
However, 6- and 7-F-benzoxazoles **12d** and **12e** were clearly less potent than **5**. Next, we evaluated **12a** and **12c** with the IC_{50} values of less than 10 nM for estimated serum concentration but found that those compounds did not demonstrate any improvement.

We next explored 4-(hetroarylcarboxamido)phenyl derivatives in which the terminal phenylamino group in compound **4** was replaced with the corresponding bicyclic heteroaryl rings while the central 4-amino-3-chlorophenyl group was fixed. In addition, introduction of a fluorine atom at the 2-position (R4) in the central benzene in compound **4** was also investigated. The results are shown in Table 3. Introduction of a 1-indoline (**13a**,**b**), a 3-indole (**13c**), a 3-indazole (**13d**), and a 3-benzoisothiazole (**13i**,**j**) as the bicyclic heteroaryl group showed potent activity with IC_{50}'s of less than 10 nM. However, somewhat of a decrease of activity in almost all the compounds except for **13c** was observed in the presence of 3% HSA. On the other hand, introduction of a 1-isoquinoline and a 3-benzoisoxazole resulted in a loss of potency (**13m**, IC_{50} = 32 nM; **13k**, IC_{50} = 128 nM). Given the high potency of **13c**, we also explored substituents at the 1-position in the indole ring. As a result, it was found that the introduction of a methyl group was well tolerated (**13e**, IC_{50} = 2.6 nM) but not of an ethyl

Table 3. Inhibitory Activity and Estimated Serum Concentration of 4-(Heteroarylcarboxamido)phenyl Derivatives **13a**−**m**

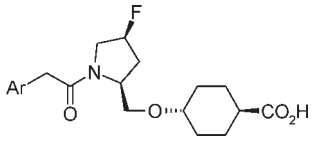


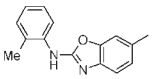
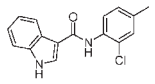
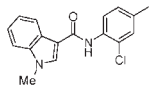
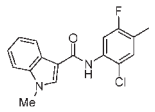
13a-m

compd	Ar	R4	IC_{50} (nM) (-/+ 3% HSA)	Estimated serum concentration (ng/mL)
13a		H	1.9 / 83	1099
13b		F	2.7 /171	2957
13c		H	0.53 / 3.9	193
13d		F	8.5 / 108	848
13e		H	2.6 / 92	5157
13f		F	5.0 / 284	11437
13g		H	204 / 21% inhibition at 1.7 μM	Not tested
13h		H	41% inhibition at 1.7 μM / 8% inhibition at 1.7 μM	Not tested
13i		H	2.7 / 196	2147
13j		F	7.5 / 176	307
13k		F	128 / 19% inhibition at 1.7 μM	Not tested
13l		H	14 / 418	Not tested
13m		F	32 / 1669	Not tested

group and an isopropyl group (**13g**, IC_{50} = 204 nM; **13h**, 41% inhibition at 1.7 μM), implying that the size of the substituent was crucial for the activity. Regarding a substituent at R4, the fluorine-substituted compounds (**13b**, **13f**, **13j**, and **13m**) were about 2−4 times less potent than the nonsubstituted compounds (**13a**, **13e**, **13i**, and **13l**). We next carried out evaluation of **13a**−**f** and **13i**−**j** for oral exposure. Among them, compounds **13a**,**b** and **13e**,**f** with no proton donor (NH) in the heteroaryl group tended to show higher oral exposure with estimated serum concentration values of more than 1000 ng/mL in comparison with compounds **13c**,**d** with the NH function, indicating that this modification would lead to a significant improvement in the

Table 4. Pharmacokinetic Properties of Compounds **5**, **13c**, **13e**, and **13f** in Rats



compd	Ar	CL (mL/min)/kg	V_{dss} (L/kg)	$T_{1/2}$ (h)
5 [a]		12.2	0.317	0.8
13c		61.1	0.668	0.2
13e		5.0	0.139	0.7
13f		2.6	0.153	0.9

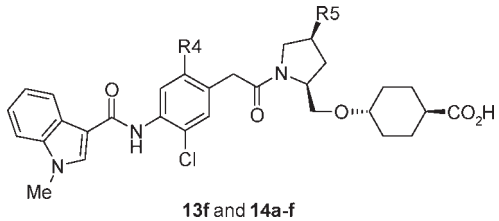
[a] Compound **5** was administered intravenously at a dose of 10 mg/kg.

pharmacokinetic properties. In particular, 1-Me-indole derivatives **13e**,**f** displayed extremely high serum concentration values of 5157 and 11437 ng/mL, respectively. Considering that the serum concentrations of **13e**,**f** were 56 and 40 times higher than the IC_{50} (+3% HSA) values of 92 and 284 nM, these compounds were expected to show efficacy *in vivo*.

Pharmacokinetic Properties of Selected 4-(3-Indolylcarboxamido)phenyl Derivatives. To investigate what the differences of the estimated serum concentration in the tested compounds were based on, we selected 4-(3-indolylcarboxamido)phenyl derivatives **13c**, **13e**,**f**, and the lead compound **5** on the basis of the serum concentration and conducted a pharmacokinetic study of these compounds using Sprague−Dawley (SD) rats (1 mg/kg, iv). The results are summarized in Table 4. As we expected, **13e**,**f** were found to clearly demonstrate low plasma clearance (**13e**, CL = 5.0 (mL/min)/kg; **13f**, CL = 2.6 (mL/min)/kg compared with **13c** and **5** (**13c**, CL = 61.1 (mL/min)/kg; **5**, CL = 12.2 (mL/min)/kg), consistent with the estimated serum concentrations. It was found that the improvement in the PK profile correlated to decrease of the PSA value of the lipophilic moiety (39.9 Å^2 for **13c**; 28.6 Å^2 for **13e**; 27.9 Å^2 for **13f**).[19] Considering that compounds **13e**,**f** with improved plasma clearance were relatively susceptible to HSA in the binding assay, this result suggests that the improved clearance of compound **13e**,**f** is at least partially a result of increased plasma protein binding.

Optimization of the (4*S*)-Substituent in the Pyrrolidine. With the 4-(1-methyl-3-indolylcarboxamido)phenyl moiety that showed the improved plasma clearance in hand, we next turned our attention to optimization of the (4*S*)-substituent on the pyrrolidine ring in **13f**. Thus, we carried out replacement of the (4*S*)-fluorine atom in **13f** with other functional groups (H, OH, OMe), which were selected based on the SAR in the (*N*′-phenylureido)phenyl derivatives we

Table 5. Inhibitory Activity and Estimated Serum Concentration of 4-(1-Methyl-3-indolylcarboxamido)phenyl Derivatives **13f** and **14a−f**



13f and **14a-f**

compd	R4	R5	IC_{50} (nM) (−/+ 3% HSA)	estimated serum concentration (ng/mL)
14a	F	H	12/370	Not tested
14b	F	OH	6.4/45	452
14c	Cl	OH	7.8/70	93
14d	F	OMe	2.9/36	9405
14e	Cl	OMe	5.4/93	6153
13f	F	F	5.0/284	11437
14f	Cl	F	5.5/241	1878

previously reported.[25] In addition, we also examined replacement of the fluorine atom with the chlorine atom at the 2-position in the central benzene in **13f**. The results are summarized in Table 5. Almost all the compounds except for **14a** (R5 = H) retained their potency with IC_{50} values of less than 10 nM. These compounds also showed more potent activity than **13f** in the presence of 3% HSA likely due to a decrease of lipophylicity. This result was in line with the SAR of a series of (*N*′-phenylureido)phenyl derivatives. On the other hand, only **14d**,**e** with R5 = MeO showed oral exposure with estimated serum concentration values of 9405 ng/mL and 6153 ng/mL, respectively.

***In Vivo* Evaluation and Safety Assessment.** On the basis of the *in vitro* activity and estimated serum concentration, we selected compounds **13e**,**f** and **14d**,**e** and conducted further biological evaluation. First, compounds **13e**,**f** and **14d**,**e** were evaluated for their anti-inflammatory effect in an *Ascaris*-antigen-induced murine asthma model by measuring the level of eosinophils in bronchoalveolar lavage fluid (BALF) at 48 h after an antigen challenge.[22] All the compounds were administered orally at three different doses (1.67, 5, and 15 mg/kg bid (twice a day) for **13e**,**f** and **14e**; 5, 15, and 45 mg/kg bid for **14d**) to mice. All the compounds (**13e**, **13f**, **14d**, and **14e**) reduced the eosinophil accumulation in a dose-dependent manner with 13%, 57%, 30%, and 53% inhibition observed at 5 mg/kg, respectively. In addition, the efficacy of compounds **13f** and **14e** (≥5 mg/kg bid) was comparable to that of the anti-mouse α_4 antibody[27] (R1-2, 5 mg/kg, sid (once a day), subcutaneous (sc), 54−62% inhibition) used as the positive control in this experiment. At present, the efficacy differences between these compounds cannot be fully explained from the activities and PK profiles, and we consider that further investigation would be necessary to make the details explicit.

Next, compounds **13f** and **14e** showing excellent efficacy were assessed for their safety profile in mice. According to the results, it was revealed that compound **13f** showed cholestatic liver injury when dosed intravenously at 50 mg/kg or orally at 25 (mg/kg)/day for 2 days. On the other hand, when compound **14e** was dosed intravenously at 50 mg/kg or orally at 200 (mg/kg)/day for 2 days, no hepatotoxicity was observed. In addition, compound **14e** demonstrated favorable results in advanced *in vitro* profiling assays with regard to CYP inhibition, microsomal stability, human ether-a-go-go related gene (hERG), and genotoxicity. Based on its good *in vivo* efficacy, safety, and ADME profiles, compound **14e** was selected as a potential clinical candidate for further pharmacological and PK evaluation.

Table 6. Selectivity of **14e** toward Other Integrins

integrin/ligand binding assay[a]	IC_{50}
VLA-4/VCAM-1[b]	5.4 nM
$\alpha_L\beta_2$/ICAM-1[c]	9% inhibition at 162 μM
$\alpha_4\beta_7$/MAdCAM-1[b]	41% inhibition at 16 μM
$\alpha_{IIb}\beta_3$[d]	10% inhibition at 16 μM

[a] The details of the binding assays are described in the Experimental Section. [b] Cell/protein binding assay. [c] Protein/protein binding assay. [d] $\alpha_{IIb}\beta_3$-dependent platelet aggregation assay.

Pharmacology of Compound 14e. Integrin Selectivity of Compound 14e. Compound **14e** was tested for its integrin selectivity in a cell/protein or protein/protein binding assay. As shown in Table 6, compound **14e** was found to show high selectivity for VLA-4 over other integrins such as $\alpha_L\beta_2$, $\alpha_4\beta_7$, and $\alpha_{IIb}\beta_3$.

Asthma Models. To evaluate the efficacy of compound **14e** in animal models, we used actively sensitized guinea pig and mouse asthma models, assessing its ability to reduce bronchial hyper-responsiveness (BHR) to acetylcholine chloride (Ach) at 24 or 48 h after an antigen challenge, respectively. In the guinea pig model, when dosed orally at 0.8−12.5 mg/kg bid, compound **14e** significantly reduced the bronchial responsiveness in a dose-dependent manner (ID_{50} = 3.0 mg/kg). Also, compound **14e**, administered orally at 12.5 mg/kg bid to mice, reduced the BHR to Ach by 83% in a murine asthma model.[26]

Pharmacokinetic Properties. The PK properties of compound **14e** were determined in rats, dogs, and monkeys. As shown in Table 7, compound **14e** exhibited favorable plasma clearance and oral bioavailability in all species.

Conclusion

In this study we optimized the PK profile of *trans*-4-substituted cyclohexanecarboxylic acid derivative **5** by modifying its lipophilic moiety. We have found that the replacement of the lipophilic moiety with a 4-(1-methyl-3-indolylcarboxamido)phenyl group was effective in improving the PK profiles in rodents while retaining VLA-4 inhibitory activity. In addition, it has been found that the improvement

Table 7. Pharmacokinetic Properties of **14e** in Rats, Dogs and Monkeys

		po			iv			
species	F^d (%)	AUC^e (ng·h/mL)	$C_{max}{}^f$ (ng/mL)	$T_{1/2}{}^g$ (h)	AUC^e (ng·h/mL)	CL^h (mL/min)/kg	$V_{dss}{}^i$ (L/kg)	$T_{1/2}{}^g$ (h)
Rat[a]	>100	975	385	1.5	865	19.3	1.222	1.0
Dog[b]	91	6091	1586	2.0	6712	1.7	0.164	2.1
Monkey[c]	68	3480	431	5.5	5141	1.8	0.223	6.3

[a] Dose: po and iv at 1 mg/kg (n = 4). [b] Dose: po and iv at 0.5 mg/kg (n = 3). [c] Dose: po and iv at 0.5 mg/kg (n = 3). [d] Oral bioavailability. [e] Pharmacokinetic area under curve. [f] Pharmacokinetic maximum concentration. [g] Plasma half-life. [h] Pharmacokinetic clearance. [i] Volume of distribution.

of the PK profile has correlated to a decrease in the polar surface area in this moiety. Through subsequent fine-tuning of substituents in the 4-(1-methyl-3-indolylcarboxamido)phenyl moiety and the pyrrolidine ring, this study culminated in the discovery of compound **14e**, *trans*-4-[1-[[2,5-dichloro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid. Compound **14e** demonstrated excellent efficacy in animal models of asthma and PK profiles in rats, dogs, and monkeys. On the basis of its favorable overall profile, compound **14e** was advanced into clinical trials for the treatment of asthma. In a single ascending-dose phase I clinical study, compound **14e** showed good oral exposure as expected from the results of PK analysis in preclinical species. In addition, there were no serious adverse events, and it was well tolerated up to 960 mg/man in this study. The detailed results of the phase I clinical study will be reported in due course.

Experimental Section

Chemistry. All starting materials and synthesis reagents were obtained commercially. 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 precoated TLC glass sheets with silica gel 60 F254. Yields were of purified products and were not optimized. Optical rotations were measured with a HORIBA SEPA-300 polarimeter. 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 high-resolution mass (HRMS) spectra were recorded on a JEOL JMS-100LP spectrometer. HPLC analysis was performed on a SHIMADZU 10A series with a Waters Symmetry C_{18} column (i.d. 4.6 mm × 250 mm) using MeCN/0.02 N NaOAc buffer (1:1, v/v) as an eluent. Elemental analysis was performed using a PerkinElmer CHNS/O 2400II, a Leco CHNS-932 and a YOKOKAWA analysis IC7000RS. Elemental data for all tested compounds are within ±0.4% of the theoretical values. Purities of ≥95% were determined by elemental analysis (all tested compounds, **11**−**14**) and HPLC (**11a**, **11b**, **11d**, **12b**, **12c**, **12e**).

General Procedure A: Preparation of *trans*-4-[(4*S*)-Fluoro-1-[[2-(2-methylbenzyl)-6-benzoxazolyl]acetyl]-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (11a). A mixture of [2-(2-methylbenzyl)-6-benzoxazolyl]acetic acid (**7a**, 131 mg, 0.47 mmol), methyl *trans*-4-[(*4S*)-fluoro-(*2S*)-pyrrolidinylmethoxy]-cyclohexanecarboxylate (**10b**, 120 mg, 0.46 mmol), EDC·HCl (134 mg, 0.70 mmol), HOBt (94 mg, 0.70 mmol), and Et_3N (97 μL, 0.70 mmol) in DMF (5 mL) was stirred at room temperature for 3 h. The mixture was diluted with H_2O and extracted with EtOAc. The extract was 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 (60:1, v/v) as an eluent to give methyl *trans*-4-[(4*S*)-fluoro-1-[[2-(2-methylbenzyl)-6-benzoxazolyl]acetyl]-(*2S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (242 mg, 99%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.17−1.31 (m, 2H), 1.38−1.52 (m, 2H), 1.96−2.28 (m, 7H), 2.39 (s, 3H), 3.21−3.51 (m, 2H), 3.61−4.02 (m, 8H), 4.24 (s, 2H), 4.19−4.38 (m, 1H), 5.14−5.29 (m, 1H), 7.15−7.19 (m, 4H), 7.28−7.29 (m, 1H), 7.39−7.40 (m, 1H), 7.57−7.61 (m, 1H); MS (ESI), m/z 523 $[M + H]^+$.

To a stirred solution of methyl *trans*-4-[(4*S*)-fluoro-1-[[2-(2-methylbenzyl)-6-benzoxazolyl]acetyl]-(*2S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (242 mg, 0.46 mmol) in THF (3 mL) was added 0.5 N NaOH (3.0 mL, 1.50 mmol), and the reaction mixture was stirred at room temperature for 2 h. The mixture was poured into ice−1 N HCl and extracted with EtOAc. The extract was 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 (60:1 to 15:1, v/v) as an eluent to give the title compound (151 mg, 64%) as a colorless amorphous solid. IR (ATR) 1720, 1610, 1433, 1095, 746 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.21−1.32 (m, 2H), 1.41−1.53 (m, 2H), 2.00−2.34 (m, 7H), 2.39 (s, 3H), 3.23−4.03 (m, 7H), 4.24 (s, 2H), 4.19−4.38 (m, 1H), 5.14−5.31 (m, 1H), 7.16−7.20 (m, 4H), 7.29−7.30 (m, 1H), 7.39−7.40 (m, 1H), 7.58−7.62 (m, 1H); MS (ESI), m/z 509 $[M + H]^+$; HRMS (ESI), m/z calcd for $C_{29}H_{33}FN_2O_5$+H, 509.2452; found, 509.2468; anal. calcd for $C_{29}H_{33}FN_2O_5 \cdot 0.5H_2O$, C 67.30, H 6.62, N 5.41; found, C 67.13, H 6.53, N 5.23; HPLC t_R = 6.5 min. (98.2%).

Compounds **11b**−**f**, **12a**−**e**, **13a**−**n**, and **14a**−**f** were prepared according to general procedure A.

***trans*-4-[1-[[7-Fluoro-2-(2-methylphenyl)amino-6-benzoxazoyl]-acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (11b).** Yield 16% (two steps). Colorless solid. IR (ATR) 2937, 2864, 1637, 1579 1454, 1070, 752 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.17−1.40 (m, 6H), 1.85−1.99 (m, 2H), 2.12−2.22 (m, 2H), 2.30 (s, 3H), 3.19 (t, J = 8.3 Hz, 1H), 3.38−4.37 (m, 8H), 5.25−5.47 (m, 1H), 6.99−7.13 (m, 3H), 7.25 (d, J = 6.8 Hz, 2H), 7.80 (d, J = 8.6 Hz, 1H), 9.87 (broad s, 1H), 12.05 (broad s, 1H). MS (ESI), m/z 528 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{28}H_{31}F_2N_3O_5$+H: 528.2310. Found: 528.2316. Anal. Calcd for $C_{28}H_{31}F_2N_3O_5 \cdot 0.2H_2O$: C, 63.31. H, 5.96; F, 7.15; N, 7.91. Found: C, 63.23; H, 5.97; F, 7.30; N, 7.80. HPLC t_R = 3.6 min. (98.7%).

***trans*-4-[1-[[2-(5-Fluoro-2-methylphenylamino)-6-benzoxazolyl]-acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (11c).** Yield 90% (two steps). Colorless solid. IR (ATR) 2939, 2864, 1639, 1610, 1576, 1437, 1242, 1097, 804 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.10−1.24 (m, 2H), 1.28−1.40 (m, 2H), 1.82−2.01 (m, 4H), 2.10−2.21 (m, 3H), 2.29 (s, 3H), 3.14−3.95 (m, 7H), 4.14−4.32 (m 1H), 5.24−5.44 (m, 1H), 6.85−6.89 (m, 1H), 7.04−7.09 (m, 1H), 7.24 (t, J = 6.8 Hz, 1H), 7.35 (t, J = 8.3 Hz, 2H), 7.95 (d, J = 11.5 Hz, 1H), 9.77 (broad s, 1H), 12.03 (broad s, 1H). MS (ESI), m/z 528 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{28}H_{31}F_2N_3O_5$+H: 528.2310. Found: 528.2306. Anal. Calcd for $C_{28}H_{31}F_2N_3O_5 \cdot 0.2H_2O$: C, 63.31; H, 5.96; F, 7.15; N, 7.91. Found: C, 63.13; H, 5.89; F, 7.15; N, 7.71.

***trans*-4-[1-[[2-(3-Fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (11d).** Yield 78% (two steps). Colorless solid. $[\alpha]_D{}^{25}$ −27.8 (c 1.0, THF). IR (ATR) 2939, 1701, 1641, 1581, 1452, 1090, 1063, 779 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.15−1.37

(m, 4H), 1.85−2.03 (m, 5H), 2.08−2.17 (m, 2H), 2.50 (d, J = 2.0 Hz, 3H), 3.19 (t, J = 8.8 Hz, 2H), 3.41−4.06 (m, 4H), 4.12−4.37 (m, 1H), 5.25−5.47 (m, 1H), 7.00 (t, J = 8.8 Hz, 1H), 6.98−7.08 (m, 2H), 7.13−7.15 (m, 1H), 7.28 (dd, J = 14.9, 8.1 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H). MS (ESI), m/z 546 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{28}H_{30}F_3N_3O_5$+H: 546.2216. Found: 546.2246. Anal. Calcd for $C_{28}H_{30}F_3N_3O_5$: C, 61.64; H, 5.54; F, 10.45; N, 7.70. Found: C, 61.40; H, 5.82; F, 10.46; N, 7.43. HPLC t_R = 6.5 min. (97.4%).

***trans*-4-[1-[[2-(4-Fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (11e).** Yield 73% (two steps). Colorless solid. $[\alpha]_D^{25}$ −28.9 (c 1.0, THF). IR (ATR) 2941, 1635, 1581, 1496, 1454, 1201, 1097, 1068, 798 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.13−1.40 (m, 4H), 1.85−2.01 (m, 4H), 2.08−2.22 (m, 3H), 2.30 (s, 3H), 3.15−3.50 (m, 2H), 3.58−4.03 (m, 5H), 4.13−4.39 (m, 1H), 5.24−5.47 (m, 1H), 6.99−7.16 (m, 4H), 7.76 (dd, J = 8.7, 5.5 Hz, 1H), 9.90 (broad s, 1H), 12.05 (broad s, 1H). MS (ESI), m/z 546 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{28}H_{30}F_3N_3O_5$+H: 546.2216. Found: 546.2244. Anal. Calcd for $C_{28}H_{30}F_3N_3O_5$: C, 61.64; H, 5.54; N, 7.70. Found: C, 61.51; H, 5.73; N, 7.41.

***trans*-4-[1-[[2-(5-Fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (11f).** Yield 68% (two steps). Colorless solid. IR (ATR) 2937, 1639, 1610, 1579, 1452, 1201, 1068, 804 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.12−1.43 (m, 4H), 1.82−2.22 (m, 7H), 2.30 (s, 3H), 3.17−3.22 (m, 1H), 3.40−4.08 (m, 6H), 4.13−4.36 (m, 1H), 5.32−5.40 (m, 1H), 6.89 (dt, J = 8.3, 2.0 Hz, 1H), 7.03−7.10 (m, 1H), 7.21 (dd, J = 8.1, 2.4 Hz, 1H), 7.26 (t, J = 7.8 Hz, 1H), 7.92 (d, J = 11.5 Hz, 1H), 10.04 (broad s, 1H). MS (ESI), m/z 546 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{28}H_{30}F_3N_3O_5$+H: 546.2216. Found: 546.2220. Anal. Calcd for $C_{28}H_{30}F_3N_3O_5 \cdot 0.4H_2O$: C, 60.84; H, 5.62; F, 10.31; N, 7.60. Found: C, 60.91; H, 5.49; F, 10.32; N, 7.41.

***trans*-4-[1-[[4-(2-Benzoxazolyl)amino-3-chlorophenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (12a).** Yield 81% (two steps). Colorless amorphous solid. $[\alpha]_D^{25}$ −36.7 (c 1.0, THF). IR (ATR) 1637, 1587, 1570, 1458, 1238, 1095, 744 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.11−1.25 (m, 2H), 1.28−1.41 (m, 2H), 1.85−1.95 (m, 4H), 2.08−2.33 (m, 3H), 3.16−3.30 (m, 2H), 3.41−3.74 (m, 3H), 3.81−3.92 (m, 2H), 4.13−4.37 (m, 1H), 5.25−5.46 (m, 1H), 7.09−7.13 (m, 1H), 7.18−7.26 (m, 2H), 7.38−7.40 (m, 2H), 7.46−7.48 (m, 1H), 7.90−7.94 (m, 1H), 9.96 (broad s, 1H), 12.03 (broad s, 1H). MS (ESI), m/z 530 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{27}H_{29}ClFN_3O_5$+H: 530.1858. Found: 530.1890. Anal. Calcd for $C_{27}H_{29}ClFN_3O_5$: C, 61.19; H, 5.52; Cl, 6.69; F, 3.58; N, 7.93. Found: C, 61.00; H, 5.40; Cl, 6.76; F, 3.65; N, 7.96.

***trans*-4-[1-[[3-Chloro-4-[2-(4-methylbenzoxazolyl)]aminophenyl]-acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (12b).** Yield 29% (two steps). Brown solid. IR (ATR) 2937, 1639, 1591, 1425, 1244, 1095, 746 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.15−1.36 (m, 4H), 1.87−2.20 (m, 7H), 2.39 (s, 3H), 3.15−3.87 (m, 7H), 4.13−4.34 (m, 1H), 5.24−5.44 (m, 1H), 6.97−7.02 (m, 2H), 7.21−7.26 (m, 2H), 7.37−7.38 (m, 1H), 7.89−7.93 (m, 1H). MS (ESI), m/z 544 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{28}H_{31}ClFN_3O_5$+H: 544.2015. Found: 544.2025. Anal. Calcd for $C_{28}H_{31}ClFN_3O_5$: C, 61.82; H, 5.74; Cl, 6.52; F, 3.49; N, 7.72. Found: C, 61.64; H, 5.87; Cl, 6.22; F, 3.37; N, 7.39. HPLC t_R = 10.7 min. (95.5%).

***trans*-4-[1-[[3-Chloro-4-[2-(5-fluorobenzoxazolyl)]aminophenyl]-acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexzanecarboxylic Acid (12c).** Yield 68% (two steps). Pale yellow solid. IR (ATR) 2937, 1637, 1589, 1566, 1442, 1136, 796 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.16−1.38 (m, 4H), 1.88−1.95 (m, 4H), 2.15−2.21 (m, 3H), 3.16−3.88 (m, 7H), 4.13−4.35 (m, 1H), 5.25−5.46 (m, 1H), 6.89−6.93 (m, 1H), 7.21−7.25 (m, 2H), 7.39−7.40 (m, 1H), 7.46−7.49 (m, 1H), 7.83−7.86 (m, 1H). MS (FAB), m/z 548 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{27}H_{28}ClF_2N_3O_5$+H: 548.1764. Found: 548.1810. Anal. Calcd for $C_{27}H_{28}ClF_2N_3O_5$: C, 59.18; H, 5.15; Cl, 6.47; F, 6.93; N, 7.54. Found: C, 59.10; H, 5.14; Cl, 6.18; F, 6.64; N, 7.41. HPLC t_R = 6.5 min. (97.1%).

***trans*-4-[1-[[3-Chloro-4-(6-fluoro-2-benzoxazolyl)aminophenyl]-acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]-1-cyclohexanecarboxylic Acid (12d).** Yield 73% (two steps). Colorless solid. IR (ATR) 1701, 1645, 1595, 1481, 1097, 957 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.10−1.34 (m, 4H), 1.83−1.92 (m, 4H), 2.09−2.19 (m, 3H), 3.14−3.88 (m, 7H), 4.11−4.31 (m, 1H), 5.22−5.42 (m, 1H), 7.04 (dt, J = 9.2, 2.4 Hz, 1H), 7.21 (dt, J = 8.0, 2.0 Hz, 1H), 7.32−7.37 (m, 2H), 7.44 (d, J = 8.0 Hz, 1H), 7.85−7.88 (m, 1H). HRMS (ESI), m/z Calcd for $C_{27}H_{28}ClF_2N_3O_5$+H: 548.1764. Found: 548.1774. Anal. Calcd for $C_{27}H_{28}ClF_2N_3O_5 \cdot 1.5H_2O$: C, 56.40; H, 5.43; N, 7.31. Found: C, 56.39; H, 5.07; N, 7.02.

***trans*-4-[1-[[3-Chloro-4-[2-(7-fluorobenzoxazolyl)]aminophenyl]-acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (12e).** Yield 62% (two steps). Brown solid. IR (ATR) 2937, 1635, 1591, 1444, 1182, 1070, 777 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.15−1.39 (m, 4H), 1.84−2.28 (m, 7H), 3.14−3.88 (m, 7H), 4.12−4.34 (m, 1H), 5.23−5.44 (m, 1H), 7.00−7.05 (m, 1H), 7.18−7.25 (m, 3H), 7.37−7.39 (m, 1H), 7.87−7.90 (m, 1H). MS (FAB), m/z 548 $[M+1]^+$. HRMS (ESI), m/z Calcd for $C_{27}H_{28}ClF_2N_3O_5$+H: 548.1764. Found: 548.1789. Anal. Calcd for $C_{27}H_{28}ClF_2N_3O_5$: C, 59.18; H, 5.15; Cl, 6.47; F, 6.93; N, 7.67. Found: C, 59.15; H, 5.08; Cl, 6.41; F, 6.76; N, 7.64. HPLC t_R = 7.1 min. (95.5%).

***trans*-4-[1-[[3-Chloro-4-(1-indolinylcarboxamido)]phenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13a).** Yield 56% (two steps). Colorless solid. IR (ATR) 2937, 1722, 1681, 1641, 1600, 1581, 1520, 1481, 1092 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.21−1.33 (m, 2H), 1.42−1.54 (m, 2H), 2.00−2.50 (m, 7H), 3.26 (t, J = 8.8 Hz, 2H), 3.24−3.36 (m, 2H), 3.48−4.00 (m, 5H), 4.14 (t, J = 8.8 Hz, 2H), 4.16−4.37 (m, 1H), 5.17−5.32 (m, 1H), 6.97 (t, J = 7.6 Hz, 1H), 7.11−7.24 (m, 4H), 7.33 (s, 1H), 7.93 (d, J = 8.0 Hz, 1H), 8.25 (t, J = 9.2 Hz, 1H). MS (ESI), m/z 558 $[M + H]^+$. HRMS (ESI), m/z Calcd for Calcd for $C_{29}H_{33}ClFN_3O_5$+H: 558.2171. Found: 558.2220. Anal. Calcd for $C_{29}H_{33}ClFN_3O_5 \cdot 0.25H_2O$: C, 61.92; H, 6.00; N, 7.47. Found: C, 62.05; H, 6.10; N, 7.17.

***trans*-4-[1-[[5-Chloro-2-fluoro-4-(1-indolinylcarboxamido)phenyl]-acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13b).** Yield 79% (two steps). Colorless amorphous solid. IR (ATR) 2941, 1712, 1675, 1642, 1535, 1485, 1113, 871, 745 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.20−1.33 (m, 2H), 1.41−1.54 (m, 2H), 2.50−2.55 (m, 7H), 3.27 (t, J = 8.0 Hz, 2H), 3.33−3.97 (m, 7H), 4.14 (t, J = 8.8 Hz, 2H), 4.29−4.38 (m, 1H), 5.19−5.36 (m, 1H), 6.99 (t, J = 7.6 Hz, 1H), 7.19−7.24 (m, 3H), 7.34−7.37 (m, 1H), 7.93 (d, J = 8.4 Hz, 1H), 8.22 (t, J = 11.6 Hz, 1H). MS (ESI), m/z 576 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{29}H_{32}ClF_2N_3O_5$+H: 576.2077. Found: 576.2103. Anal. Calcd for $C_{29}H_{32}ClF_2N_3O_5$: C, 60.47; H, 5.60; Cl, 6.15; F, 6.60; N, 7.29. Found: C, 60.38; H, 5.57; Cl, 6.03; F, 6.38; N, 7.34.

***trans*-4-[1-[[3-Chloro-4-(3-indolylcarboxamido)phenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid. (13c).** Yield 74% (two steps). Colorless solid. IR (ATR) 3423, 3210, 2940, 2863, 1697, 1627, 1514, 1433, 1101, 735 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.13−1.41 (m, 4H), 1.86−1.96 (m, 4H), 2.11−2.21 (m, 3H), 3.16−3.28 (m, 2H), 3.42−3.88 (m, 5H), 4.14−4.36 (m, 1H), 5.26−5.46 (m, 1H), 7.13−7.21 (m, 3H), 7.39 (dd, J = 7.3, 1.8 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.64 (dd, J = 7.8, 4.6 Hz, 1H), 8.14 (d, J = 7.3 Hz, 1H), 8.29 (d, J = 2.8 Hz, 1H), 9.33 (s, 1H), 11.76 (s, 1H), 12.06 (broad s, 1H). MS (ESI), m/z 556 $[M + H]^+$. Anal. Calcd for $C_{29}H_{31}ClFN_3O_5 \cdot 1.25H_2O$: C, 60.20; H, 5.84; Cl, 6.13; F, 3.28; N, 7.26. Found: C, 60.12; H, 5.57; Cl, 6.38; F, 3.37; N, 7.37.

***trans*-4-[1-[[5-Chloro-2-fluoro-4-(3-indazolylcarboxamido)phenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13d).** Yield 25% (two steps). Colorless amorphous solid. IR (ATR) 2937, 1670, 1618, 1589, 1535, 1406, 1092, 779 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.11−1.41 (m,

4H), 1.86−2.02 (m, 4H), 2.10−2.27 (m, 3H), 3.17−3.30 (m, 2H), 3.42−3.52 (m, 1H), 3.61−3.95 (m, 4H), 4.14−4.39 (m, 1H), 5.25−5.49 (m, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.48−7.53 (m, 2H), 7.72 (d, J = 8.3 Hz, 1H), 8.12 (dd, J = 11.5, 4.9 Hz, 1H), 8.22 (d, J = 8.3 Hz, 1H), 9.79 (s, 1H), 12.04 (broad s, 1H). MS (ESI), m/z 575 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{28}H_{29}ClF_2N_4O_5$+H: 575.1873. Found: 575.1908. Anal. Calcd for $C_{28}H_{29}ClF_2N_4O_5$: C, 58.49; H, 5.08; Cl, 6.17; F, 6.61; N, 9.74. Found: C, 58.29; H, 5.13; Cl, 6.08; F, 6.51; N, 9.55.

***trans*-4-[1-[[3-Chloro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetyl-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13e).** Yield 72% (two steps). Colorless solid. IR (ATR) 2938, 2863, 1647, 1512, 1465, 1101, 744 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.19−1.39 (m, 4H), 1.90−2.11 (m, 4H), 2.14−2.27 (m, 3H), 3.17−3.22 (m, 1H), 3.43−3.87 (m, 6H), 3.89 (s, 3H), 4.16−4.35 (m, 1H), 5.25−5.45 (m, 1H), 7.18−7.28 (m, 3H), 7.37−7.39 (m, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.69−7.73 (m, 1H), 8.15 (d, J = 7.8 Hz, 1H), 8.27 (d, J = 7.8 Hz, 1H), 9.23 (s, 1H), 11.95 (broad s, 1H). MS (ESI), m/z 570 $[M + H]^+$. Anal. Calcd for $C_{30}H_{33}ClFN_3O_5 \cdot 0.75\ H_2O$: C, 61.75; H, 5.96; N, 7.20. Found: C, 61.85; H, 5.92; N, 6.83.

***trans*-4-[1-[[5-Chloro-2-fluoro-4-(1-methyl-3-indolylcarboxamido)-phenyl]acetyl-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13f).** Yield 86% (two steps). Colorless solid. IR (ATR) 2940, 2863, 1652, 1521, 1404, 1227, 1099, 744 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.16−1.41 (m, 4H), 1.86−2.32 (m, 7H), 3.17−3.84 (m, 6H), 3.89 (s, 3H), 4.13−4.38 (m, 1H), 5.25−5.47 (m, 1H), 7.19−7.29 (m, 2H), 7.41−7.46 (m, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.70−7.72 (m, 1H), 8.15 (d, J = 7.6 Hz, 1H), 8.29−8.30 (m, 1H), 9.28 (s, 1H), 12.01 (broad s, 1H). MS (ESI), m/z 588 $[M + H]^+$. Anal. Calcd for $C_{30}H_{32}ClF_2N_3O_5$: C, 61.27; H, 5.48; N, 7.15. Found: C, 61.41; H, 5.48; N, 7.11.

***trans*-4-[1-[[3-Chloro-4-(1-ethyl-3-indolylcarboxamido)phenyl]-acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13g).** Yield 77% (two steps). Colorless amorphous solid. IR (ATR) 2937, 1647, 1512, 1209, 1093, 746 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.10−1.40 (m, 4H), 1.45 (t, J = 7.2 Hz, 3H), 1.80−2.04 (m, 4H), 2.04−2.36 (m, 3H), 3.10−3.97 (m, 7H), 4.07−4.39 (m, 1H), 4.29 (q, J = 7.2 Hz, 2H), 5.32−5.39 (m, 1H), 7.10−7.30 (m, 3H), 7.38 (d, J = 6.8 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.65 (m, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.35 (s, 1H), 9.31 (s, 1H), 12.05 (broad s, 1H). MS (ESI), m/z 584 $[M + H]^+$. Anal. Calcd for $C_{31}H_{35}ClFN_3O_5 \cdot 0.25H_2O$: C, 63.26; H, 6.08; Cl, 6.02; F, 3.23; N, 7.14. Found: C, 63.20; H, 6.08; Cl, 5.86; F, 3.18; N, 6.96.

***trans*-4-[1-[[3-Chloro-4-(1-isopropyl-3-indolylcarboxamido)phenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13h).** Yield 76% (two steps). Colorless amorphous solid. IR (ATR) 2933, 1643, 1511, 1203, 1095, 746 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.08−1.44 (m, 4H), 1.53 (d, J = 6.8 Hz, 6H), 1.80−2.05 (m, 4H), 2.05−2.34 (m, 3H), 3.12−3.90 (m, 7H), 4.14−4.40 (m, 1H), 4.83−4.86 (m, 1H), 5.32−5.46 (m, 1H), 7.12−7.28 (m, 3H), 7.39 (d, J = 6.8 Hz, 1H), 7.59−7.63 (m, 2H), 8.17 (d, J = 8.0 Hz, 1H), 8.48 (s, 1H), 9.35 (s, 1H), 12.04 (broad s, 1H). MS (ESI), m/z 598 $[M + H]^+$. Anal. Calcd for $C_{31}H_{37}ClFN_3O_5 \cdot 0.25H_2O$: C, 63.78; H, 6.27; Cl, 5.88; F, 3.15; N, 6.97. Found: C, 63.62; H, 6.32; Cl, 5.72; F, 3.13; N, 6.77.

***trans*-4-[1-[[4-(3-Benzo[*d*]isothiazolylcarboxamido)-3-chloro-phenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13i).** Yield 64% (two steps). Colorless solid. IR (ATR) 2935, 2862, 1691, 1604, 1579, 1520, 1191, 1090, 742 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.11−1.38 (m, 4H), 1.86−1.99 (m, 4H), 2.12−2.33 (m, 3H), 3.17−3.89 (m, 7H), 4.14−4.36 (m, 1H), 5.25−5.46 (m, 1H), 7.26 (t, J = 6.4 Hz, 1H), 7.44 (dd, J = 7.7, 1.6 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H), 7.69−7.73 (m, 1H), 7.93−7.96 (m, 1H), 8.37 (d, J = 8.3 Hz, 1H), 8.80 (d, J = 8.1 Hz, 1H), 10.27 (s, 1H), 12.05 (broad s, 1H). MS (ESI), m/z 575 $[M + H]^+$. Anal. Calcd for $C_{28}H_{29}ClFN_3O_5S$: C, 58.58; H, 5.09; N, 7.32. Found: C, 58.41; H, 5.13; N, 7.02.

***trans*-4-[1-[[4-(3-Benzo[*d*]isothiazolylcarboxamido)-5-chloro-2-fluorophenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13j).** Yield 64% (two steps). Colorless solid. IR (ATR) 2935, 1691, 1637, 1622, 1525, 1406, 1196, 1093, 741 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.17−1.38 (m, 4H), 1.82−2.23 (m, 7H), 3.20−4.02 (m, 7H), 4.13−4.38 (m, 1H), 5.35−5.49 (m, 1H), 7.50−7.55 (m, 1H), 7.64−7.74 (m, 2H), 7.93−7.97 (m, 1H), 8.38 (d, J = 8.0 Hz, 1H), 8.81 (d, J = 8.3 Hz, 1H), 10.30 (s, 1H), 12.05 (broad s, 1H). MS (ESI), m/z 592 $[M + H]^+$. Anal. Calcd for $C_{28}H_{28}ClF_2N_3O_5S \cdot 0.5H_2O$: C, 55.95; H, 4.86; N, 6.99. Found: C, 56.01; H, 4.84; N, 6.88.

***trans*-4-[1-[[4-(3-Benzo[*d*]isoxazolecarboxamido)-5-chloro-2-fluorophenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13k).** Yield 61% (two steps). Colorless solid. IR (ATR) 2937, 1736, 1697, 1622, 1533, 1097, 756 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.19−1.40 (m, 4H), 1.88−2.23 (m, 7H), 3.19−4.00 (m, 7H), 4.13−4.38 (m, 1H), 5.36−5.49 (m, 1H), 7.50−7.58 (m, 2H), 7.66−7.68 (m, 1H), 7.77−7.81 (m, 1H), 7.94 (d, J = 8.3 Hz, 1H), 8.16 (d, J = 8.1 Hz, 1H), 10.65 (broad s, 1H), 12.05 (broad s, 1H). MS (ESI), m/z 576 $[M + H]^+$. Anal. Calcd for $C_{28}H_{28}ClF_2N_3O_6$: C, 58.39; H, 4.90; N, 7.30. Found: C, 58.19; H, 4.87; N, 7.18.

***trans*-4-[1-[[3-Chloro-4-(1-isoquinolinylcarboxamido)phenyl]-acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (13l).** Yield 84% (two steps). Yellow solid. IR (ATR) 2935, 2861, 1716, 1596, 1527, 1442, 1093, 754 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.13−1.41 (m, 4H), 1.85−2.25 (m, 7H), 3.17−3.89 (m, 7H), 4.14−4.36 (m, 1H), 5.25−5.46 (m, 1H), 7.26−7.30 (m, 1H), 7.43−7.45 (m, 1H), 7.81 (t, J = 7.1 Hz, 1H), 7.88 (t, J = 7.1 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 5.6 Hz, 1H), 8.20−8.24 (m, 1H), 8.67 (d, J = 5.6 Hz, 1H), 9.31 (d, J = 8.5 Hz, 1H), 10.84 (s, 1H), 12.02 (broad s, 1H). MS (ESI), m/z 568 $[M + H]^+$. Anal. Calcd for $C_{30}H_{31}ClFN_3O_5$: C, 63.43; H, 5.50; N, 7.40. Found: C, 63.40; H, 5.62; N, 7.13.

***trans*-4-[1-[[5-Chloro-2-fluoro-4-(1-isoquinolinylcarboxamido)-phenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclozhexanecarboxylic Acid (13m).** Yield 87% (two steps). Yellow solid. IR (ATR) 3282, 2940, 2863, 1722, 1693, 1650, 1619, 1581, 1517 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.15−1.41 (m, 4H), 1.86−2.33 (m, 7H), 3.17−4.00 (m, 7H), 4.14−4.37 (m, 1H), 5.26−5.48 (m, 1H), 7.49−7.54 (m, 1H), 7.82 (t, J = 7.3 Hz, 1H), 7.88 (t, J = 7.3 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 8.18−8.23 (m, 2H), 8.67 (d, J = 5.6 Hz, 1H), 9.38 (d, J = 8.8 Hz, 1H), 10.95−10.96 (m, 1H), 12.02 (broad s, 1H). MS (ESI), m/z 586 $[M + H]^+$. Anal. Calcd for $C_{30}H_{30}ClF_2N_3O_5 \cdot 0.25H_2O$: C, 61.02; H, 5.21; N, 7.12. Found: C, 61.01; H, 5.17; N, 7.00.

***trans*-4-[1-[[5-Chloro-2-fluoro-4-(1-methyl-3-indolylcarboxamido)-phenyl]acetyl]-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (14a).** Yield 74% (two steps). $[\alpha]_D^{25}$ −49.9 (c 1.0, $CHCl_3$). Colorless amorphous solid. IR (ATR) 2933, 1724, 1612, 1514, 1404, 1219, 1182, 1099, 744 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.13−1.28 (m, 2H), 1.40−1.53 (m, 2H), 1.88−2.06 (m, 8H), 2.23−2.34 (m, 1H), 3.16−3.25 (m, 1H), 3.37−3.80 (m, 6H), 3.88 (s, 3H), 4.13−4.25 (m, 1H), 7.32−7.37 (m, 2H), 7.39−7.44 (m, 2H), 7.81 (s, 1H), 8.11−8.14 (m, 1H), 8.29 (s, 1H), 8.46−8.51 (m, 1H). MS (FAB), m/z 570 $[M + H]^+$. Anal. Calcd for $C_{30}H_{33}ClFN_3O_5 \cdot 0.25H_2O$: C, 62.71; H, 5.88; N, 7.31. Found: C, 62.55; H, 5.99; N, 6.96.

***trans*-4-[1-[[5-Chloro-2-fluoro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetyl]-(4*S*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]-cyclohexanecarboxylic Acid (14b).** Yield 75% (two steps). Pale yellow solid. IR (ATR) 2935, 1633, 1520, 1400, 1227, 1099, 742 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.14−1.37 (m, 4H), 1.75−2.17 (m, 7H), 3.10−3.93 (m, 7H), 3.88 (s, 3H), 4.01−4.31 (m, 2H), 5.04−5.09 (m, 1H), 7.19−7.29 (m, 2H), 7.40−7.45 (m, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.67−7.71 (m, 1H), 8.14 (d, J = 7.8 Hz, 1H), 8.30 (s, 1H), 9.29 (s, 1H), 12.03 (broad s, 1H). MS (ESI), m/z 586 $[M + H]^+$. Anal. Calcd for $C_{30}H_{33}ClFN_3O_6 \cdot 0.25H_2O$: C, 61.01; H, 5.72; Cl, 6.00; F, 3.22; N, 7.12. Found: C, 61.04; H, 5.75; Cl, 6.06; F, 3.15; N, 6.95.

***trans*-4-[1-[[2,5-Dichloro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetyl]-(4*S*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (14c).** Yield 56% (two steps). Colorless solid. IR (ATR) 2937, 1716, 1630, 1500, 1373, 1219, 1101, 1076, 744 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.13−1.36 (m, 4H), 1.79−2.18 (m, 7H), 3.10−3.95 (m, 7H), 3.88 (s, 3H), 4.00−4.35 (m, 2H), 5.04−5.13 (m, 1H), 7.20 (t, J = 7.3 Hz, 1H), 7.26 (t, J = 7.3 Hz, 1H), 7.47−7.55 (m, 2H), 7.87−7.88 (m, 1H), 8.14 (d, J = 7.6 Hz, 1H), 8.29 (s, 1H), 9.36 (s, 1H). MS (ESI), m/z 602 $[M + H]^+$. Anal. Calcd for $C_{30}H_{33}Cl_2N_3O_6 \cdot 1.25H_2O$: C, 57.65; H, 5.72; N, 6.72. Found: C, 57.53; H, 5.61; N, 6.44.

***trans*-4-[1-[[5-Chloro-2-fluoro-4-(1-methyl-3-indolylcarboxamido)-phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (14d).** Yield 96% (two steps). Colorless solid. IR (ATR) 2937, 1701, 1664, 1626, 1587, 1522, 1402, 1217, 1097, 742 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.12−1.42 (m, 4H), 1.82−2.19 (m, 7H), 3.14−3.19 (m, 2H), 3.23 and 3.26 (each s, total 3H, amide isomers), 3.44−3.83 (m, 5H), 3.89 (s, 3H), 3.94−4.25 (m, 2H), 7.22 (t, J = 7.3 Hz, 1H), 7.27 (t, J = 6.8 Hz, 1H), 7.42−7.45 (m, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.68−7.70 (m, 1H), 8.15 (d, J = 7.8 Hz, 1H), 8.31 (d, J = 2.2 Hz, 1H), 9.31 (s, 1H). MS (ESI), m/z 600 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{31}H_{35}ClFN_3O_6$+H: 600.2277. Found: 600.2300. Anal. Calcd for $C_{31}H_{35}ClFN_3O_6 \cdot 0.8H_2O$: C, 60.59; H, 6.00; N, 6.84. Found: C, 60.60; H, 5.97; N, 6.74.

***trans*-4-[1-[[2,5-Dichloro-4-(1-methyl-3-indolylcarboxamido)-phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (14e).** Yield 69% (two steps). Colorless fine needles. $[\alpha]_D^{25}$ −33.5 (*c* 1.1, THF). IR (ATR) 2939, 1728, 1600, 1498, 1379, 1216, 1174, 1100, 1086, 1076, 743 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.11−1.40 (m, 4H), 1.88−2.20 (m, 7H), 3.14−3.51 (m, 5H), 3.58−3.82 (m, 4H), 3.89 (s, 3H), 3.92−4.26 (m, 3H), 7.21 (t, J = 7.3 Hz, 1H), 7.28 (t, J = 6.9 Hz, 1H), 7.49−7.53 (m, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.88−7.90 (m, 1H), 8.15 (d, J = 7.8 Hz, 1H), 8.31 (s, 1H), 9.39 (d, J = 2.8 Hz, 1H), 12.06 (broad s, 1H). MS (ESI), m/z 616 $[M + H]^+$. HRMS (ESI), m/z Calcd for $C_{31}H_{35}Cl_2N_3O_6$+H: 616.1981. Found: 616.2003. Anal. Calcd for $C_{31}H_{35}Cl_2N_3O_6 \cdot 0.25H_2O$: C, 59.95; H, 5.76; Cl, 11.42; N, 6.77. Found: C, 59.68; H, 5.64; Cl, 11.57; N, 6.80.

***trans*-4-[1-[[2,5-Dichloro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (14f).** Yield 75% (two steps). Colorless solid. IR (ATR) 2937, 2864, 1726, 1651, 1500, 1379, 1221, 1101, 1076, 741 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.15−1.38 (m, 4H), 1.85−2.01 (m, 4H), 2.11−2.33 (m, 3H), 3.18−3.23 (m, 1H), 3.46−3.97 (m, 6H), 3.89 (s, 3H), 4.13−4.37 (m, 1H), 5.26−5.49 (m, 1H), 7.21 (t, J = 7.4 Hz, 1H), 7.28 (t, J = 7.4 Hz, 1H), 7.50 (d, J = 15.7 Hz, 1H), 7.55 (d, J = 8.1 Hz, 1H), 7.88 (d, J = 3.4 Hz, 1H), 8.14 (d, J = 7.8 Hz, 1H), 8.30 (s, 1H), 9.38 (s, 1H), 12.04 (broad s, 1H). HRMS (ESI), m/z Calcd for $C_{30}H_{32}Cl_2FN_3O_5$+H: 604.1781. Found: 604.1821. Anal. Calcd for $C_{30}H_{32}Cl_2FN_3O_5 \cdot 0.25H_2O$: C, 59.17; H, 5.38; N, 6.90. Found: C, 59.18; H, 5.38; N, 6.71.

[2-(2-Methylbenzyl)-6-benzoxazolyl]acetic Acid (7a). To a cooled (0 °C), stirred solution of *o*-tolylacetic acid (414 mg, 2.76 mmol), methyl (4-amino-3-hydroxyphenyl)acetate (**15**, 500 mg, 2.76 mmol), Ph_3P (1.81 g, 6.90 mmol), and Et_3N (1.92 mL, 13.8 mmol) in MeCN (20 mL) was added dropwise a solution of hexachloroethane (1.44 g, 6.08 mmol) in CH_2Cl_2 (6 mL) for 5 min. The reaction mixture was stirred at room temperature for 20 h. After being filtered through a Celite pad, the filtrate was evaporated. The residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (3:1, v/v) as an eluent to give methyl [2-(2-methylbenzyl)-6-benzoxazolyl]acetate (190 mg, 23%) as a pale yellow oil. ^1H NMR ($CDCl_3$) δ 2.38 (s, 3H), 3.68 (s, 3H), 3.71 (s, 2H), 4.24 (s, 2H), 7.18−7.25 (m, 4H), 7.27−7.29 (m, 1H), 7.39−7.40 (m, 1H), 7.59−7.62 (m, 1H). MS (ESI), m/z 296 $[M + H]^+$.

To a stirred solution of methyl [2-(2-methylbenzyl)-6-benzoxazolyl]acetate (190 mg, 0.64 mmol) in THF (4 mL) was added 0.5 N NaOH (3.9 mL, 1.95 mmol), and the reaction mixture was stirred at room temperature for 2 h. The mixture was poured into ice−1 N HCl. The resulting precipitate was collected by suction and dried under vacuum to give the title compound (131 mg, 72%) as a pale yellow solid. ^1H NMR (DMSO-d_6) δ 2.28 (s, 3H), 3.67 (s, 2H), 4.30 (s, 2H), 7.13−7.26 (m, 5H), 7.53−7.58 (m, 2H).

Dimethyl (3-Benzyloxy-2-fluoro-4-nitrophenyl)malonate (17). To a cooled (0 °C), stirred solution of 2,3-difluoro-6-nitrophenol (**16**, 314.5 g, 1.79 mol) in DMF (3 L) was added dropwise benzyl bromide (239.6 mL, 2.02 mol) and K_2CO_3 (273.6 g, 1.98 mol). The reaction mixture was stirred at room temperature for 48 h. After being filtered by suction, the filtrate was poured into H_2O. The mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was recrystallized from *n*-hexane/EtOAc to give 2-benzyloxy-3,4-difluoro-1-nitrobenzene (380.2 g, 80%) as a pale yellow solid. ^1H NMR (DMSO-d_6) δ 5.30 (s, 2H), 7.36−7.49 (m, 6H), 7.87−7.92 (m, 1H).

To a cooled (−4 °C), stirred solution of dimethyl malonate (339.1 g, 2.57 mol) in NMP (2 L) was added NaH (60% in oil, 136.8 g, 3.42 mol) for 40 min under nitrogen atmosphere. To the mixture was added 2-benzyloxy-3,4-difluoro-1-nitrobenzene (453.7 g, 1.71 mol) in NMP (775 mL) at −4 °C for 1 h, and the reaction mixture was stirred at 45 °C for 2 h. After being cooled to room temperature, the mixture was poured into H_2O. After being made acidic (pH = 3) with 2 N HCl, the mixture was extracted with EtOAc. The extract was 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 (5:1 to 3:1, v/v) as an eluent to give the title compound (659.6 g, 100%) as a pale yellow oil. ^1H NMR (DMSO-d_6) δ 3.74 (s, 6H), 5.21 (s, 2H), 5.40 (s, 1H), 7.35−7.42 (m, 6H), 7.79 (dd, J = 8.7, 1.8 Hz, 1H). MS (ESI), m/z 378 $[M + H]^+$.

Methyl (3-Benzyloxy-2-fluoro-4-nitrophenyl)acetate (18). To a stirred solution of **17** (659.6 g, 1.75 mol) in MeOH (2.6 L) was added 2 N NaOH (2.6 L, 5.20 mol), and the reaction mixture was heated under reflux for 3 h. After being cooled to room temperature, the mixture was made acidic (pH = 4) by the addition of conc. HCl. The resulting solid was collected by suction, washed with H_2O, and dried under vacuum to give (3-benzyloxy-2-fluoro-4-nitrophenyl)acetic acid (465.0 g, 89%) as a colorless solid. ^1H NMR (DMSO-d_6) δ 3.79 (d, J = 1.8 Hz, 2H), 5.20 (s, 2H), 7.30−7.44 (m, 6H), 7.73 (dd, J = 8.5, 1.6 Hz, 1H).

To a stirred solution of (3-benzyloxy-2-fluoro-4-nitrophenyl)acetic acid (465.0 g, 1.52 mol) in MeOH (5 L) was added conc. H_2SO_4 (150 mL), and the reaction mixture was heated under reflux for 4 h. After being cooled to room temperature, the mixture was concentrated to a small volume. The residue was diluted with H_2O and extracted with $CHCl_3$. The extract was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo to give the title compound (486.3 g, 100%) as a pale yellow oil. ^1H NMR ($CDCl_3$) δ 3.74 (s, 2H), 3.75 (s, 3H), 5.24 (s, 2H), 7.10 (dd, J = 8.5, 6.7 Hz, 1H), 7.33−7.41 (m, 3H), 7.46−7.49 (m, 2H), 7.59 (dd, J = 8.7, 1.8 Hz, 1H). MS (ESI), m/z 320 $[M + H]^+$.

Methyl (4-Amino-2-fluoro-3-hydroxyphenyl)acetate (19). A suspension of **18** (486.3 g, 1.52 mmol) and 5% Pd/C (wet) (48.6 g) in MeOH (5 L) was stirred at room temperature under hydrogen atmosphere for 14 h. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (3:1 to 2:1, v/v) as an eluent to give the title compound (238.2 g, 78%) as a light brown solid. ^1H NMR ($CDCl_3$) δ 3.57 (s, 2H), 3.70 (s, 3H), 6.45−6.47 (m, 1H), 6.57−6.61 (m, 1H).

[2-(3-Fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]-acetic Acid (7d). To a stirred solution of 3-fluoro-2-methylaniline (0.57 mL, 5.0 mmol) in THF (20 mL) was added thiocarbonyl diimidazole (990 mg, 5.0 mmol) at room temperature. After

4 h stirring, **19** (996 mg, 5.0 mmol) was added to the mixture, and the reaction mixture was stirred for 2 days. HgO (1.08 g, 5.0 mmol) was added to the reaction mixture and heated at 70 °C for 4.5 h. After being cooled to room temperature, the reaction mixture was filtered through a Celite pad, and the filtered cake was washed with MeOH. The filtrate was concentrated in vacuo and the residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (4:1, v/v) as an eluent to give methyl [2-(3-fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]acetate (1.21 g, 73%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H), 3.72 (s, 3H), 3.76 (s, 2H), 6.87 (t, J = 8.8 Hz, 1H), 7.09 (t, J = 6.6 Hz, 1H), 7.22−7.27 (m, 2H), 7.93 (d, J = 7.8 Hz, 1H). MS (ESI), m/z 333 $[M + H]^+$.

To a stirred solution of methyl [2-(3-fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]acetate (1.21 g, 3.64 mmol) in THF/MeOH (2:1, v/v, 60 mL) was added 1 N NaOH (20 mL). After stirring at room temperature for 17 h, the mixture was concentrated in vacuo and acidified with 1 N HCl. The resulting precipitate was collected, washed with water, and dried under reduced pressure to give the title compound (1.10 g, 15%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.21 (d, J = 2.0 Hz, 3H), 3.66 (s, 2H), 7.00 (t, J = 8.8 Hz, 1H), 7.10−7.16 (m, 2H), 7.26−7.30 (m, 1H), 7.72 (d, J = 7.1 Hz, 1H), 10.10 (broad s, 1H). MS (ESI), m/z 319 $[M + H]^+$.

General Procedure B: Preparation of [2-(5-Fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]acetic Acid (7f). To a stirred solution of **19** (1.0 g, 5.02 mmol) in MeOH (30 mL) was added 5-fluoro-2-methylphenyl isothiocyanate (1.0 g, 5.98 mmol) at room temperature. After 5 days stirring, HgO (1.14 g, 4.36 mmol) was added to the reaction mixture, and the mixture was heated at 70 °C for 6 h. After being cooled to room temperature, the reaction mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$/EtOAc (30:1, v/v) as an eluent to give methyl [2-(5-fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]acetate (810 mg, 56%) as a pink solid. ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H), 3.72 (s, 3H), 3.76 (s, 2H), 6.75 (dt, J = 8.1, 2.7 Hz, 1H), 6.87 (broad s, 1H), 7.09−7.17 (m, 2H), 7.24−7.25 (m 1H), 8.11 (dd, J = 11.0, 6.7 Hz, 1H). MS (ESI), m/z 333 $[M + H]^+$.

To a stirred solution of methyl [2-(5-fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]acetate (810 mg, 2.44 mmol) in THF/MeOH (90 mL, 2:1, v/v) was added 1 N NaOH (30 mL). After being stirred at room temperature for 12 h, the mixture was concentrated under reduced pressure and acidified with 1 N HCl. The precipitate was collected, washed with water, and dried under reduced pressure to give the title compound (700 mg, 90%) as a pale brown solid. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 3.68 (s, 2H), 6.89 (dt, J = 7.0, 1.7 Hz, 1H), 7.14 (t, J = 6.4 Hz, 1H), 7.20−7.27 (m, 2H), 7.90 (d, J = 11.3 Hz, 1H), 10.06 (broad s, 1H). MS (ESI), m/z 319 $[M + H]^+$.

Compounds **7b**, **7c**, and **7e** were prepared according to general procedure B.

[7-Fluoro-2-(2-methylphenylamino)-6-benzoxazolyl]acetic Acid (7b). Yield 86% (two steps). Light brown solid. ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 3.68 (s, 2H), 7.09−7.18 (m, 3H), 7.25 (d, J = 6.9 Hz, 2H), 7.80 (d, J = 8.1 Hz, 1H), 8.30 (s, 1H), 12.46 (broad s, 1H). MS (ESI), m/z 301 $[M + H]^+$.

[2-(5-Fluoro-2-methylphenylamino)-6-benzoxazolyl]acetic Acid (7c). Yield 45% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 3H), 3.64 (s, 2H), 6.87 (dt, J = 8.3, 1.5 Hz, 1H), 7.11 (d, J = 8.1 Hz, 1H), 7.25 (t, J = 7.3 Hz, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.41 (s, 1H), 7.96 (d, J = 11.5 Hz, 1H), 9.80 (broad s, 1H). MS (ESI), m/z 301 $[M + H]^+$.

[2-(4-Fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]-acetic Acid (7e). Yield 91% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H), 3.68 (s, 2H), 7.06−7.14 (m, 4H), 7.73−7.76 (m, 1H), 9.91 (s, 1H). MS (ESI), m/z 319 $[M + H]^+$.

Ethyl [4-(2-Benzoxazolyl)amino-3-chlorophenyl]acetate (21). A mixture of 2-chlorobenzoxazole (743 μL, 6.51 mmol) and ethyl (4-amino-3-chlorophenyl)acetate (**20a**, 1.30 g, 6.51 mmol) in xylene (10 mL) was heated under reflux for 2 h. After being cooled to room temperature, the mixture was diluted with $CHCl_3$. The mixture was washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (9:1, v/v) as an eluent to give the title compound (1.70 g, 79%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.25−1.28 (m, 3H), 3.58 (s, 2H), 4.14−4.19 (m, 2H), 7.15−7.19 (m, 1H), 7.24−7.30 (m, 3H), 7.36−7.38 (m, 2H), 7.52−7.54 (m, 1H), 8.51−8.53 (m, 1H). MS (ESI), m/z 331 $[M + H]^+$.

[4-(2-Benzoxazolyl)amino-3-chlorophenyl]acetic Acid (8a). To a stirred solution of **21** (1.70 g, 5.14 mmol) in THF (30 mL) was added 0.5 N NaOH (30 mL, 15.0 mmol), and the reaction mixture was stirred at room temperature for 20 h. The mixture was concentrated to a small volume and poured into ice−1 N HCl. The resulting precipitate was collected by suction and dried under vacuum to give the title compound (1.24 g, 80%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 2H), 7.10−7.19 (m, 1H), 7.21−7.28 (m, 1H), 7.30−7.31 (m, 1H), 7.38−7.40 (m, 1H), 7.45−7.49 (m, 3H), 7.94−7.96 (m, 1H).

Methyl (3-Chloro-4-isothiocyanatophenyl)acetate (22). To a cooled (0 °C), stirred suspension of $CaCO_3$ (626 mg, 6.25 mmol) and thiophosgene (191 μL, 2.51 mmol) in CH_2Cl_2−H_2O (10 mL, 1:1, v/v) was added methyl (4-amino-3-chlorophenyl)acetate (**20b**, 500 mg, 2.50 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at 0 °C to room temperature for 1.5 h. To the mixture was added 1 N HCl, and the mixture was extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and evaporated to give the title compound (652 mg, 100%) as a yellow oil. MS (ESI) m/z 241 $[M]^+$.

General procedure C: Preparation of [4-[2-(4-Methylbenzoxazolyl)]amino-3-chlorophenyl]acetic Acid (8b). A mixture of **22** (652 mg, 2.50 mmol) and 2-amino-3-methylphenol (**23a**, 307 mg, 2.50 mmol) in toluene (15 mL) was heated under reflux for 2 h. Then HgO (541 mg, 2.50 mmol) was added to the mixture, and the reaction mixture was heated under reflux for 5 h. After being cooled to room temperature, the mixture was filtered through a Celite pad, and the filtrate was evaporated. The residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (7:1, v/v) as an eluent to give methyl [4-[2-(4-methylbenzoxazolyl)]amino-3-chlorophenyl]acetate (359 mg, 43%) as a black oil. ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3H), 3.58 (s, 2H), 3.70 (s, 3H), 7.02−7.06 (m, 2H), 7.11−7.19 (m, 1H), 7.25−7.28 (m, 1H), 7.33−7.34 (m, 1H), 7.50 (broad s, 1H), 8.54−8.56 (m, 1H). MS (ESI), m/z 331 $[M + H]^+$.

To a stirred solution of methyl [4-[2-(4-methylbenzoxazolyl)]amino-3-chlorophenyl]acetate (359 mg, 1.08 mmol) in THF (6 mL) was added 0.5 N NaOH (6.5 mL, 3.25 mmol), and the reaction mixture was stirred at room temperature for 4 h. The mixture was poured into ice−1 N HCl and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated to give the title compound (281 mg, 82%) as a brown solid. ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3H), 3.57 (s, 2H), 6.72−6.74 (m, 1H), 7.04−7.06 (m, 2H), 7.18−7.20 (m, 2H), 8.46−8.48 (m, 1H). MS (ESI), m/z 317 $[M + H]^+$.

Compounds **8c**−**e** were prepared according to general procedure C.

[3-Chloro-4-(5-fluoro-2-benzoxazolyl)aminophenyl]acetic Acid (8c). Yield 7% (two steps). Brown solid. ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 2H), 6.89−6.94 (m, 1H), 7.21−7.30 (m, 2H), 7.45−7.49 (m, 2H), 7.85−7.87 (m, 1H), 10.16 (broad s, 1H), 12.44 (broad s, 1H). MS (ESI), m/z 321 $[M + H]^+$.

[3-Chloro-4-(6-fluoro-2-benzoxazolyl)aminophenyl]acetic Acid (8d). Yield 14% (two steps). Pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.55 (s, 2H), 7.02−7.07 (m, 1H), 7.26 (dd, J = 8.4, 2.4 Hz, 1H), 7.35 (dd, J = 8.0, 4.4 Hz, 1H), 7.42 (d, J = 1.6 Hz, 1H), 7.47 (dd,

J = 8.4, 2.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 10.0 (broad s, 1H).

[3-Chloro-4-(7-fluoro-2-benzoxazolyl)aminophenyl]acetic Acid (8e). Yield 11% (2 steps). Brown solid. MS (ESI), m/z 321 $[M + H]^+$.

General procedure D: Preparation of [3-Chloro-4-(1-indolinylcarboxamido)phenyl]acetic Acid (9a). To a cooled (0 °C), stirred solution of **20a** (2.00 g, 9.36 mmol) in CH_2Cl_2 (50 mL) was added triphosgene (926 mg, 3.12 mmol) and pyridine (5 mL). After the mixture was stirred at room temperature for 15 h, indoline (1.05 mL, 9.36 mmol) was added to the mixture, and the reaction mixture was stirred at room temperature for 15 h. The mixture was diluted with H_2O and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and evaporated to give ethyl [3-chloro-4-(1-indolinylcarboxamido)phenyl]acetate (3.07 g, 91%) as a light pink crystalline powder. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H), 3.20−3.26 (m, 2H), 3.54 (d, J = 2.7 Hz, 2H), 4.08−4.18 (m, 4H), 6.93−6.98 (m, 1H), 7.09−7.31 (m, 5H), 7.94 (d, J = 8.1 Hz, 1H), 8.28 (d, J = 8.1 Hz, 1H). MS (ESI), m/z 359 $[M + H]^+$.

To a stirred solution of ethyl [3-chloro-4-(1-indolinylcarboxamido)phenyl]acetate (3.07 g, 8.56 mmol) in THF (70 mL) was added 0.25 N NaOH (68.4 mL, 17.1 mmol), and the reaction mixture was heated under reflux for 4 h. After being cooled to room temperature, the mixture was poured into 1 N HCl (50 mL). The resulting precipitate was collected by suction and dried under vacuum to give the title compound (1.71 g, 60%) as a colorless crystalline powder. ^{1}H NMR (DMSO-d_6) δ 3.16 (t, J = 8.8 Hz, 2H), 3.57 (s, 2H), 4.11 (t, J = 8.8 Hz, 2H), 6.86 (t, J = 7.3 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 7.13−7.20 (m, 2H), 7.38 (s, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 8.3 Hz, 1H), 8.15 (s, 1H), 12.36 (s, 1H). MS (ESI), m/z 331 $[M + H]^+$.

Compound **9b** was prepared according to general procedure D.

[5-Chloro-2-fluoro-4-(1-indolinylcarboxamido)phenyl]acetic Acid (9b). Yield 94% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.19 (t, J = 8.4 Hz, 2H), 3.63 (s, 2H), 4.15 (t, J = 8.8 Hz, 2H), 6.91 (t, J = 7.6 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 7.20 (d, J = 7.6 Hz, 1H), 7.51 (d, J = 7.2 Hz, 1H), 7.59 (d, J = 11.2 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 8.19 (s, 1H), 12.50 (broad s, 1H).

General procedure E: Preparation of 1-Ethylindole-3-carboxylic Acid (27d). To a stirred suspension of NaH (60% in oil, 274 mg, 6.85 mmol) in DMF (8 mL) was added methyl indole-3-carboxylate (**25a**, 400 mg, 2.28 mmol) at 0 °C, and the mixture was stirred for 40 min. To the mixture was added ethyl iodide (0.27 mL, 3.42 mmol), and the mixture was stirred at 0 °C for 2 h. The reaction mixture was quenched by the addition of H_2O and extracted with EtOAc. The extract was washed with 1 N HCl, dried over Na_2SO_4, and evaporated to give methyl 1-ethylindole-3-carboxylate (**26a**), which was used in the subsequent reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.52 (t, J = 7.2 Hz, 3H), 3.91 (s, 3H), 4.20 (q, J = 7.2 Hz, 2H), 7.23−7.28 (m, 2H), 7.33−7.38 (m, 1H), 7.84 (s, 1H), 8.15−8.20 (m, 1H). MS (ESI), m/z 204 $[M + H]^+$.

To a stirred solution of **26a** in THF (9 mL) was added 0.25 N NaOH (13 mL) at room temperature. The reaction mixture was stirred at 50 °C for 9 h. After being cooled to room temperature, the mixture was neutralized with 1 N HCl, and the resulting precipitate was collected by suction, washed with water, and dried at 50 °C to give the title compound (388 mg, 90% for two steps) as a pale pink amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.38 (t, J = 7.2 Hz, 3H), 4.26 (q, J = 7.2 Hz, 2H), 7.12−7.28 (m, 2H), 7.55 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 8.07 (s, 1H), 11.93 (broad s, 1H).

Compound **27e** was prepared according to general procedure E.

Methyl 1-Isopropylindole-3-carboxylate (26b). Yield 78%. Light yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.56 (d, J = 6.8 Hz, 6H), 3.91 (s, 3H), 4.62−4.72 (m, 1H), 7.24−7.29 (m, 2H), 7.36−7.40 (m, 1H), 7.95 (s, 1H), 8.16−8.20 (m, 1H). MS (ESI), m/z 218 $[M + H]^+$.

1-Isopropylindole-3-carboxylic Acid (27e). Yield 90%. Light pink amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.50 (d, J = 6.6 Hz, 6H), 4.77−4.82 (m, 1H), 7.17−7.25 (m, 2H), 7.61 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 7.6 Hz, 1H), 8.13 (s, 1H), 11.97 (broad s, 1H).

General Procedure F: Preparation of [5-Chloro-2-fluoro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetic Acid (9f). To a cooled (0 °C), stirred solution of 1-methylindole-3-carboxylic acid (**27c**, 1.0 g, 5.71 mmol) in CH_2Cl_2 (10 mL) was added $(COCl)_2$ (735 μL, 8.57 mmol), and the reaction mixture was stirred at room temperature for 10 min. After removal of the solvent, the residue was dissolved in CH_2Cl_2 (20 mL). To the mixture was added ethyl (4-amino-5-chloro-2-fluorophenyl)acetate (**20c**, 1.32 g, 5.71 mmol) and Et_3N (1.60 mL, 11.4 mmol), and the reaction mixture was heated under reflux for 17 h. After being cooled to room temperature, the mixture was diluted with water and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with n-hexane/EtOAc (2:1, v/v) as an eluent to give ethyl [5-chloro-2-fluoro-4-(1-methyl-3-indolylcarboxamido)phenyl]-acetate (1.55 g, 70%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7.3 Hz, 3H), 3.62 (s, 2H), 3.88 (s, 3H), 4.19 (q, J = 7.3 Hz, 2H), 7.32−7.43 (m, 4H), 7.80 (s, 1H), 8.11−8.16 (m, 1H), 8.29 (broad s, 1H), 8.53 (d, J = 12.0 Hz, 1H). MS (ESI), m/z 389 $[M + H]^+$.

To a stirred solution of ethyl [5-chloro-2-fluoro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetate (1.55 g, 3.99 mmol) in THF (30 mL) was added 0.25 N NaOH (32 mL, 8.0 mmol), and the reaction mixture was stirred at room temperature for 17 h. After the solution was acidified with 1 N HCl, the resulting precipitate was collected by suction and dried under vacuum to give the title compound (1.33 g, 92%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.66 (s, 2H), 3.89 (s, 3H), 7.20−7.29 (m, 2H), 7.55 (d, J = 7.8 Hz, 2H), 7.73−7.78 (m, 1H), 8.15 (d, J = 7.8 Hz, 1H), 8.31 (s, 1H), 9.29 (s, 1H), 12.56 (broad s, 1H). MS (ESI), m/z 361 $[M + H]^+$.

Compounds **9e**−**i** were prepared according to general procedure F.

[3-Chloro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetic Acid (9e). Yield 28% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.61 (s, 2H), 3.89 (s, 3H), 7.17−7.28 (m, 3H), 7.43 (d, J = 1.7 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 8.14 (d, J = 7.8 Hz, 1H), 8.26 (s, 1H), 9.26 (s, 1H).

[2,5-Dichloro-4-(1-methyl-3-indolecarboxamido)phenyl]acetic Acid (9g). Yield 71%. Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 2H), 3.90 (s, 3H), 7.22 (t, J = 8.1 Hz, 1H), 7.28 (t, J = 8.1 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.64 (s, 1H), 7.92 (s, 1H), 8.15 (d, J = 7.8 Hz, 1H), 8.31 (s, 1H), 9.39 (s, 1H). MS (ESI), m/z 378 $[M + H]^+$.

[3-Chloro-4-(1-ethyl-3-indolylcarboxamido)phenyl]acetic Acid (9h). Yield 83%. Brown amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.43 (m, 3H), 3.62 (s, 2H), 4.28 (m, 2H), 7.12−7.30 (m, 2H), 7.44 (m, 1H), 7.58 (m, 1H), 7.66 (m, 1H), 7.91−8.10 (m, 1H), 8.15 (m, 1H), 8.35 (m, 1H), 9.31 (s, 1H). MS (ESI), m/z 357 $[M + H]^+$.

[3-Chloro-4-(1-isopropyl-3-indolylcarboxamido)phenyl]acetic Acid (9i). Yield 85%. Brown amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.52 (d, J = 6.4 Hz, 6H), 3.62 (s, 2H), 4.77−4.89 (m, 1H), 7.13−7.30 (m, 3H), 7.44 (s, 1H), 7.60−7.64 (m, 2H), 8.12−8.20 (m, 1H), 8.48 (s, 1H), 9.36 (s, 1H), 12.32 (broad s, 1H). MS (ESI), m/z 371 $[M + H]^+$.

General Procedure G: Preparation of [3-Chloro-4-(3-indolylcarboxamido)phenyl]acetic Acid (9c). To a stirred solution of indole-3-carboxylic acid (**27a**, 1.00 g, 6.21 mmol), **20a** (1.33 g, 6.22 mmol) and Et_3N (1.80 mL, 12.9 mmol) in DMF (24 mL) was added EDC·HCl (1.78 g, 9.28 mmol), and the reaction mixture was stirred at 70 °C for 17 h. After being cooled to room

temperature, the mixture was diluted with H_2O and extracted with EtOAc. The extract was 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 (4:1 to 1:1, v/v) as an eluent to give ethyl [3-chloro-4-(3-indolylcarboxamido)phenyl]acetate (1.25 g, 56%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.1 Hz, 3H), 3.59 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 7.23−7.25 (m, 1H), 7.31−7.38 (m, 3H), 7.45−7.49 (m, 1H), 7.92 (d, J = 2.9 Hz, 1H), 8.15−8.18 (m, 1H), 8.32 (broad s, 1H), 8.58 (d, J = 8.5 Hz, 1H), 8.78 (broad s, 1H). MS (ESI), m/z 357 $[M + H]^+$.

To a stirred solution of ethyl [3-chloro-4-(3-indolylcarboxamido)phenyl]acetate (1.25 g, 3.50 mmol) in THF (35 mL) was added 0.25 N NaOH (21 mL, 5.23 mmol), and the reaction mixture was stirred at room temperature for 4 h. After being concentrated to a small volume, the residue was made acidic with 1 N HCl (10 mL). The resulting solid was collected by suction, washed with H_2O, and dried under vacuum to give the title compound (1.05 g, 91%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.61 (s, 2H), 7.12−7.20 (m, 2H), 7.24 (d, J = 8.3 Hz, 1H), 7.44 (s, 1H), 7.47 (d, J = 7.3 Hz, 1H), 7.67 (d, J = 8.3 Hz, 1H), 8.14 (d, J = 7.3 Hz, 1H), 8.29 (s, 1H), 9.32 (s, 1H), 11.75 (broad s, 1H).

Compounds **9j**−**n** were prepared according to general procedure G.

[4-(Benzo[*d*]-3-isothiazolylcarboxamido)-3-chlorophenyl]acetic Acid (9j). Yield 92% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.59 (s, 2H), 7.30 (dd, J = 8.2, 1.8 Hz, 1H), 7.49 (d, J = 1.7 Hz, 1H), 7.61−7.66 (m, 1H), 7.69−7.73 (m, 1H), 7.94 (d, J = 8.3 Hz, 1H), 8.36 (d, J = 8.1 Hz, 1H), 8.80 (d, J = 8.1 Hz, 1H), 10.26 (s, 1H). MS (ESI), m/z 347 $[M + H]^+$.

[5-Chloro-2-fluoro-4-(benzo[*d*]-3-isothiazolylcarboxamido)phenyl]acetic Acid (9k). Yield 30% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 2H), 7.65 (d, J = 7.3 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 7.73 (t, J = 7.5 Hz, 1H), 7.99 (d, J = 11.2 Hz, 1H), 8.38 (d, J = 8.3 Hz, 1H), 8.81 (d, J = 7.8 Hz, 1H), 10.30 (s, 1H). MS (ESI), m/z 365 $[M + H]^+$.

[4-(Benzo[*d*]-3-isoxazolylcarboxamido)-5-chloro-2-fluorophenyl]acetic Acid (9l). Yield 10% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.66 (s, 2H), 7.54−7.57 (m, 1H), 7.61−7.63 (m, 1H), 7.67−7.70 (m, 1H), 7.78−7.81 (m, 1H), 7.93−7.95 (m, 1H), 8.15−8.17 (m, 1H), 10.62 (s, 1H). MS (ESI), m/z 349 $[M + H]^+$.

[3-Chloro-4-(1-isoquinolinylcarboxamido)phenyl]acetic Acid (9m). Yield 55% (two steps). Brown solid. ^{1}H NMR (DMSO-d_6) δ 3.63 (s, 2H), 7.33 (dd, J = 8.3, 1.7 Hz, 1H), 7.51 (d, J = 1.7 Hz, 1H), 7.81 (td, J = 8.3, 1.7 Hz, 1H), 7.88 (td, J = 8.3, 1.7 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 5.6 Hz, 1H), 8.24 (d, J = 8.3 Hz, 1H), 8.67 (d, J = 5.6 Hz, 1H), 9.34 (d, J = 8.3 Hz, 1H), 10.85 (s, 1H), 12.48 (broad s, 1H). MS (ESI), m/z 341 $[M + H]^+$.

[5-Chloro-2-fluoro-4-(1-isoquinolinylcarboxamido)phenyl]acetic Acid (9n). Yield 75%. Pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.66 (s, 2H), 7.62 (d, J = 7.5 Hz, 1H), 7.80−7.90 (m, 2H), 8.12 (d, J = 8.5 Hz, 1H), 8.18 (d, J = 5.6 Hz, 1H), 8.23 (d, J = 11.5 Hz, 1H), 8.67 (d, J = 5.6 Hz, 1H), 9.38 (d, J = 8.5 Hz, 1H), 10.98 (s, 1H), 12.59 (broad s, 1H). MS (ESI), m/z 359 $[M + H]^+$.

Methyl 1-(4-Methoxybenzyl)-3-indazolylcarboxylate (26c). To a stirred solution of methyl 3-indazolylcarboxylate (**25b**, 2.0 g, 11.4 mmol) in DMF (20 mL) was added 4-methoxybenzyl chloride (1.7 mL, 12.5 mmol) and K_2CO_3 (2.35 g, 17.0 mmol), and the reaction mixture was stirred at 80 °C for 15 h. After being cooled to room temperature, the mixture was poured into H_2O and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and 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 (more polar fractions, 838 mg, 25%) as a pale yellow oil and 2-PMB isomer (less polar fractions, 500 mg, 15%) as a pale yellow oil. For the title compound: ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H), 4.05 (s, 3H), 5.64 (s, 2H), 6.82 (d, J = 8.5 Hz, 2H), 7.20 (d, J = 8.5 Hz, 2H), 7.30 (dd, J = 8.1, 4.2 Hz, 1H), 7.36−7.37 (m, 2H), 8.23 (dd, J = 8.1, 1.0 Hz, 1H). MS (ESI), m/z 297 $[M + H]^+$.

1-(4-Methoxybenzyl)-3-indazolylcarboxylic Acid (27b). To a stirred solution of **26c** (830 mg, 2.80 mmol) in THF (22 mL) was added 0.25 N NaOH (22 mL, 5.60 mmol), and the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was poured into 1 N HCl and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in $CHCl_3$, and hexane was added until a precipitate formed. The resulting precipitate was collected by suction and dried under vacuum to give the title compound (630 mg, 80%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 3H), 5.69 (s, 2H), 6.88 (d, J = 8.8 Hz, 2H), 7.26 (d, J = 8.8 Hz, 2H), 7.29−7.32 (m, 1H), 7.43−7.48 (m, 1H), 7.84 (d, J = 8.6 Hz, 1H), 8.08 (d, J = 8.6 Hz, 1H), 13.06 (broad s, 1H). MS (ESI), m/z 283 $[M + H]^+$.

[5-Chloro-2-fluoro-4-(3-indazolylcarboxamido)phenyl]acetic Acid (9d). To a stirred suspension of **27b** (630 mg, 2.23 mmol) in CH_2Cl_2 (10 mL) were added $(COCl)_2$ (292 μL, 3.53 mmol) and DMF (1 drop). The mixture was stirred until the suspension turned into a clear solution. The resulting solution was concentrated in vacuo to remove excess $(COCl)_2$. The solid obtained was dissolved in CH_2Cl_2 (20 mL), and ethyl (4-amino-5-chloro-2-fluorophenyl)acetate (**20c**, 517 mg, 2.23 mmol) and Et_3N (621 μL, 4.46 mmol) were added. The mixture was heated under reflux for 15 h. After being cooled to room temperature, the mixture was quenched by the addition of H_2O and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and 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 ethyl [5-chloro-2-fluoro-4-[1-(4-methoxybenzyl)-3-indazolylcarboxamido]phenyl]-acetate (881 mg, 80%) as a pale yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7.1 Hz, 3H), 3.62 (s, 2H), 3.77 (s, 3H), 4.19 (q, J = 7.1 H, 2H), 5.59 (s, 2H), 6.85 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.8 Hz, 2H), 7.24−7.42 (m, 4H), 8.39 (d, J = 8.1 Hz, 1H), 8.54 (d, J = 11.7 Hz, 1H), 9.56 (s, 1H). MS (ESI), m/z 496 $[M + H]^+$.

To a stirred solution of ethyl [5-chloro-2-fluoro-4-[1-(4-methoxybenzyl)-3-indazolylcarboxamido]phenyl]acetate (880 mg, 1.77 mmol) in TFA (10 mL) was added anisole (288 μL, 2.66 mmol), and the reaction mixture was heated under reflux for 15 h. After being cooled to room temperature, the mixture was concentrated in vacuo. The residue was diluted with H_2O, and the resulting precipitate was collected by suction. The solid was dissolved in $CHCl_3$−MeOH, and *n*-hexane was added until a precipitate formed. The precipitate was collected by suction and dried under vacuum to give ethyl [5-chloro-2-fluoro-4-(3-indazolylcarboxamido)phenyl]acetate (531 mg, 80%) as a colorless crystalline powder. ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.1 Hz, 3H), 3.75 (s, 2H), 4.12 (q, J = 7.1 Hz, 2H), 7.34 (t, J = 7.8 Hz, 1H), 7.49 (dt, J = 8.1, 1.0 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 11.5 Hz, 1H), 8.21 (d, J = 8.1 Hz, 1H), 9.79 (s, 1H). MS (ESI), m/z 376 $[M + H]^+$.

To a stirred solution of ethyl [5-chloro-2-fluoro-4-(3-indazolylcarboxamido)phenyl]acetate (531 mg, 1.41 mmol) in MeOH (50 mL) was added 0.25 N NaOH (12 mL, 3 mmol), and the reaction mixture was stirred at room temperature for 15 h. The mixture was poured into 1 N HCl, and the resulting precipitate was collected by suction and dried under vacuum to give the title compound (480 mg, 98%) as a colorless crystalline powder. ^{1}H NMR (DMSO-d_6) δ 3.63 (s, 2H), 7.34 (t, J = 7.6 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.62 (m, 1H), 7.72 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 11.2 Hz, 1H), 8.21 (d, J = 8.1 Hz, 1H), 9.79 (s, 1H), 13.96 (broad s, 1H).

Methyl 4-[*N*-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (29a). To a stirred solution of methyl 4-[*N*-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylmethoxy]benzoate[25] (**28a**, 2.47 g, 7.36 mmol) in CH_2Cl_2 (60 mL) was added

TFA (30 mL), and the reaction mixture was stirred at room temperature for 3 h. After being concentrated in vacuo, the residue was dissolved into MeOH/AcOH (55 mL, 10:1, v/v), 5% Rh on alumina (1.00 g) was added, and the reaction mixture was hydrogenated at 10 atm at room temperature for 20 h. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo. The residue was dissolved into $CHCl_3$ and washed with sat. $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to give methyl 4-[(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (1.71 g, 96% for two steps) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.20−1.53 (m, 4H), 1.62−2.09 (m, 9H), 2.23−2.39 (m, 1H), 2.82−2.88 (m, 1H), 2.95−3.02 (m, 1H), 3.19−3.48 (m, 4H), 3.66 (s, 3H). MS (ESI), *m*/*z* 242 $[M + H]^+$.

To a stirred solution of methyl 4-[(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (1.71 g, 7.09 mmol) and Et_3N (1.50 mL, 10.6 mmol) in $MeCN/H_2O$ (40 mL, 1:1, v/v) was added Boc_2O (1.70 g, 7.80 mmol), and the reaction mixture was stirred at room temperature for 20 h. The mixture was diluted with EtOAc, washed with 1 N HCl and brine, dried over $MgSO_4$, and 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 (2.26 g, 93%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.21−1.53 (m, 4H), 1.46 (s, 9H), 1.61−2.05 (m, 7H), 2.26−2.37 (m, 1H), 3.20−3.74 (m, 6H), 3.66 (s, 3H), 3.82−3.92 (m, 1H).

Methyl *trans*-4-[*N*-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30a). To a stirred solution of **29a** (2.26 g, 6.62 mmol) in MeOH (50 mL) was added NaOMe (1.07 g, 19.9 mmol), and the reaction mixture was heated under reflux for 15 h. After being cooled to room temperature, the mixture was poured into 1 N HCl (100 mL) and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in benzene/MeOH (60 mL, 4:1, v/v) and treated with $TMSCHN_2$ (2 M in *n*-hexane, 1.70 mL, 3.40 mmol) until the carboxylic acid had disappeared on TLC. The mixture was concentrated in vacuo, and the residue was purified by flash column chromatography (Biotage 75M) with *n*-hexane/EtOAc (7:1 to 4:1, v/v) as an eluent to give the title compound (780 mg, 35%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.18−1.30 (m, 2H), 1.41−1.50 (m, 2H), 1.46 (s, 9H), 1.76−2.07 (m, 7H), 2.24−2.29 (m, 1H), 3.18−3.40 (m, 5H), 3.57−3.63 (m, 1H), 3.66 (s, 3H), 3.79−3.94 (m, 1H).

Methyl *trans*-4-[(2*S*)-Pyrrolidinylmethoxy]cyclohexanecarboxylate (10a). To a stirred solution of **30a** (780 mg, 2.28 mmol) in CH_2Cl_2 (5 mL) was added TFA (5 mL), and the reation mixture was stirred at room temperature for 15 h. After being concentrated to a small volume, the residue was made basic with sat. $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated in vacuo to give the title compound (504 mg, 91%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.20−1.29 (m, 2H), 1.34−1.51 (m, 3H), 1.66−2.10 (m, 7H), 2.23−2.31 (m, 1H), 2.83−2.89 (m, 1H), 2.95−3.01 (m, 1H), 3.20−3.28 (m, 2H), 3.32−3.36 (m, 1H), 3.44−3.48 (m, 1H), 3.66 (s, 3H). MS (ESI), *m*/*z* 242 $[M + H]^+$.

Methyl 4-[*N*-(*tert*-Butoxycarbonyl)-(4*R*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (29c). To a stirred solution of methyl 4-[*N*-(*tert*-butoxycarbonyl)-(4*R*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]benzoate[25] (**28c**, 4.01 g, 11.4 mmol) in CH_2Cl_2 (60 mL) was added TFA (30 mL), and the reaction mixture was stirred at room temperature for 3 h. After being concentrated in vacuo, the residue was dissolved into EtOH (80 mL). To the mixture was added 5% Rh on alumina (1.01 g), and the reaction mixture was hydrogenated at 10 atm at room temperature for 20 h. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo. The residue was dissolved into $CHCl_3$/MeOH (10:1, v.v), washed with sat. $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo to give methyl 4-[(4*R*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (2.46 g, 86% for two steps) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.20−1.85 (m, 6H), 1.99−2.40 (m, 5H), 3.30−3.75 (m, 6H), 3.65 (s, 3H), 4.17 (broad s, 1H), 4.65 (broad s, 1H).

To a stirred solution of methyl 4-[(4*R*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (1.41 g, 5.48 mmol) and Et_3N (1.53 mL, 10.98 mmol) in CH_2Cl_2 (30 mL) was added Boc_2O (1.25 g, 5.73 mmol), and the reaction mixture was stirred at room temperature for 1 h. After being concentrated in vacuo, the residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (15:1, v/v) as an eluent to give the title compound (1.95 g, 100%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.11−1.85 (m, 6H), 1.46 (s, 9H), 1.95−2.05 (m, 3H), 2.12−2.36 (m, 2H), 3.17−3.59 (m, 6H), 3.66 (s, 3H) 4.03 (broad s, 1H), 4.47−4.51 (m, 1H). MS (ESI), *m*/*z* 358 $[M + H]^+$.

Methyl 4-[(4*R*)-Benzyloxymethoxy-*N*-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (29d). To a stirred solution of **29c** (1.37 g, 3.83 mmol) and diisopropylethylamine (1.0 mL, 5.75 mmol) in CH_2Cl_2 (20 mL) was added benzylchloromethyl ether (796 μL, 5.75 mmol), and the reaction mixture was stirred at room temperature for 17 h. The mixture was diluted with H_2O and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$/EtOAc (10:1, v/v) as an eluent to give the title compound (1.45 g, 79%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.35−1.66 (m, 4H), 1.45 (s, 9H), 1.78−1.86 (m, 4H), 2.05−2.22 (m, 2H), 2.33 (broad s, 1H), 3.44−3.73 (m, 5H), 3.66 (s, 3H), 3.97−4.04 (m, 1H), 4.40 (broad s, 1H), 4.57−4.64 (m, 2H), 4.78 (s, 2H), 7.28−7.45 (m, 5H).

Methyl *trans*-4-[(4*R*)-Benzyloxymethoxy-*N*-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30c). To a stirred solution of **29d** (1.45 g, 3.04 mmol) in MeOH (50 mL) was added NaOMe (493 mg, 9.12 mmol), and the reaction mixture was heated under reflux for 2 days under N_2 atmosphere. After being cooled to room temperature, the mixture was quenched by the addition of 1 N HCl and extracted with $CHCl_3$/MeOH (5:1, v/v). The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in benzene/MeOH (50 mL, 4:1, v/v). To the solution was added $TMSCHN_2$ (2 M in *n*-hexane, 2.0 mL, 4.0 mmol), and the reaction mixture was stirred at room temperature for 30 min. The mixture was quenched by the addition of AcOH and concentrated in vacuo. The residue was purified by flash column chromatography (Biotage 40M) with *n*-hexane/EtOAc (7:1 to 4:1, v/v) as an eluent to give the title compound (669 mg, 46%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.18−1.28 (m, 2H), 1.39−1.50 (m, 2H), 1.46 (s, 9H), 1.97−2.28 (m, 7H), 3.16−3.23 (m, 1H), 3.39−3.63 (m, 4H), 3.66 (s, 3H), 3.93−4.04 (m, 1H), 4.34−4.40 (m, 1H), 4.56−4.63 (m, 2H), 4.77 (s, 2H), 7.28−7.37 (m, 5H). MS (ESI), *m*/*z* 478 $[M + H]^+$.

Methyl *trans*-4-[*N*-(*tert*-Butoxycarbonyl)-(4*R*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30d). A mixture of **30c** (669 mg, 1.40 mmol) and 5% Pd/C (600 mg) in MeOH (50 mL) was stirred at room temperature under hydrogen atmosphere for 20 h. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo to give the title compound (500 mg, 100%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.16−1.30 (m, 2H), 1.41−1.50 (m, 2H), 1.46 (s, 9H), 1.95−2.05 (m, 5H), 2.12−2.18 (m, 1H), 2.23−2.29 (m, 1H), 3.16−3.23 (m, 1H), 3.34−3.62 (m, 5H), 3.66 (s, 3H), 4.02 (broad s, 1H), 4.44−4.49 (m, 1H).

Methyl *trans*-4-[*N*-(*tert*-Butoxycarbonyl)-(4*S*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30e). To a stirred soloution of **30d** (5.00 g, 14.0 mmol), Ph_3P (4.41 g, 16.8 mmol), and formic acid (1.58 mL, 42.0 mmol) in THF (50 mL) was added diisopropyl azodicarboxylate (DIAD, 3.30 mL, 16.8 mmol), and the reaction mixture was stirred at room temperature for 20 h. The mixture was concentrated in vacuo, and the residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (4:1, v/v) as an eluent to give methyl *trans*-4-[*N*-(*tert*-butoxycarbonyl)-(4*S*)-formyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (5.39 g) as a colorless oil. To a stirred solution of the product in THF (50 mL) was added sat. $NaHCO_3$ (50 mL), and the reaction mixture was stirred at room

temperature for 20 h. After being concentrated to a small volume, the residue was diluted with H_2O and extracted with EtOAc. The extract was 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 (1:1, v/v) as an eluent to give the title compound (4.08 g, 82% for two steps) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.24−1.34 (m, 2H), 1.42−1.52 (m, 2H), 1.46 (s, 9H), 1.86−1.92 (m, 1H), 1.99−2.09 (m, 4H), 2.24−2.36 (m, 2H), 3.30−3.36 (m, 1H), 3.41−3.54 (m, 3H), 3.66 (s, 3H), 3.92−4.21 (m, 3H), 4.83−5.07 (m, 1H). MS (ESI), *m*/*z* 358 $[M + H]^+$.

Methyl *trans*-4-[(4*S*)-Hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (10c). To a stirred solution of **30e** (57 mg, 0.16 mmol) in CH_2Cl_2 (3 mL) was added TFA (1 mL), and the reation mixture was stirred at room temperature for 5 h. After being concentrated to a small volume, the residue was made basic with sat. $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give the title compound (38 mg, 92%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.26−1.36 (m, 2H), 1.43−1.54 (m, 2H), 1.79−1.82 (m, 1H), 1.99−2.16 (m, 4H), 2.24−2.34 (m, 2H), 3.04−3.12 (m, 1H), 3.25−3.28 (m, 1H), 3.39−3.48 (m, 1H), 3.61−3.78 (m, 3H), 3.67 (s, 3H), 4.37 (broad s, 1H). MS (ESI), *m*/*z* 258 $[M + H]^+$.

Methyl *trans*-4-[*N*-(*tert*-Butoxycarbonyl)-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30f). To a cooled (0 °C), stirred solution of **30e** (3.00 g, 8.39 mmol) and MeI (2.60 mL, 42.0 mmol) in DMF (20 mL) was added NaH (60% in oil) (336 mg, 8.39 mmol), and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was poured into ice 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 column chromatography on silica gel with *n*-hexane/EtOAc (2:1, v/v) as an eluent to give the title compound (1.82 g, 59%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.18−1.28 (m, 2H), 1.40−1.49 (m, 2H), 1.46 (s, 9H), 1.94−2.08 (m, 5H), 2.15−2.28 (m, 2H), 3.17−3.25 (m, 1H), 3.30 (s, 3H), 3.33−3.56 (m, 3H), 3.66 (s, 3H), 3.69−3.81 (m, 1H), 3.88−3.96 (m, 2H). MS (ESI), *m*/*z* 372 $[M + H]^+$.

Methyl *trans*-4-[(4*S*)-Methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (10d). To a stirred solution of **30f** (50 mg, 0.135 mmol) in CH_2Cl_2 (5 mL) was added TFA (0.5 mL), and the reaction mixture was stirred at room temperature for 14 h. After being concentrated to a small volume, the mixture was made basic by the addition of 1 N NaOH and extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and concentrated to give the title compound (37 mg, 100%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.22−1.35 (m, 2H), 1.41−1.57 (m, 2H), 1.96−2.20 (m, 5H), 2.24−2.35 (m, 2H), 3.33 (s, 3H), 3.34−3.57 (m, 4H), 3.66 (s, 3H), 3.67−3.81 (m, 2H), 3.90−3.96 (m, 1H), 4.11−4.15 (m, 1H). MS (ESI), *m*/*z* 272 $[M + H]^+$.

Biology. All protein labeled with europium (Eu) was prepared by using Eu-labeling reagent (PerkinElmer Inc.), and purified using a PD-10 column (Amersham Biosciences KK.). Eu-labeled protein was stored at −80 °C until use.

VLA-4/Eu-Human VCAM-1 Binding Assay. A human VLA-4-expressing cell line, 4B4, was established at Pharmacopeia Inc. by transfecting both the α_4 gene and β_1 gene of VLA-4 into CHO-K1 cells. The 4B4 cells were maintained in Ham's F-12 medium (Sigma Corp.) supplemented with 10% (v/v) fetal calf serum (REHATUIN fetal bovine serum, Serologicals Corp.), 100 U/mL penicillin (Invitrogen Corp.), 100 μg/mL streptomycin (Invitrogen Corp.), 2 mM L-glutamine (Invitrogen Corp.), and 1 mg/mL G-418 (Geneticin, Invitrogen Corp.). A Eu-labeling reagent (PerkinElmer Inc.) was used to labeled the human VCAM-1/Fc chimeria (R&D Systems Inc.). All assays were performed in duplicate. In preparation for the assay, the 4B4 cells were suspended at 3×10^5 cells/mL in Ham's F-12 medium. One hundred microliters of the 4B4 cell suspension was placed into each well of a 96-well-culture plate (Costar Inc.). The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 2 days. Prior to the assay, the medium was discarded, and each well was washed twice with 300 μL of chilled wash buffer (25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.5; 150 mM NaCl; 1 mM $CaCl_2$; 1 mM $MgCl_2$; 4 mM $MnCl_2$). Then, 50 μL of compound solution was added to a well, followed by 50 μL of 2 nM Eu-labeled human VCAM-1/Fc chimera diluted with the wash buffer (final concentration 1 nM). For assays conducted in the presence of human serum albumin, 50 μL of compound at various concentrations and an equal volume of 2 nM Eu-labeled human VCAM-1/Fc chimera in 6% (w/v) human serum albumin (Sigma Corp.) were distributed into each well (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 (PerkinElmer Inc.) was added to each well. The plates were placed on a shaker for 5 min. Eu fluorescence was then measured using a time-resolved fluorometer (DELFIA Research fluorometer, model 1234-001; PerkinElmer Inc.). The concentration of compound required for 50% inhibition in the assay was determined.

$\alpha_4\beta_7$/Eu-Human MAdCAM-1 Binding Assay. RPMI8866, a human B cell line expressing $\alpha_4\beta_7$ but not β_1 integrin, was maintained in medium, RPMI1640 including 10% FCS (fetal calf serum), Pn/St. For the RPMI8866/MAdCAM-1 binding assays, RPMI8866 cells were washed and resuspended in assay buffer (0.1% BSA, 20 μM diethylene triamine pentaacetic acid (DTPA), 25 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM $MnCl_2$) and prepared at 3.0×10^7 cells/mL (final cell number 1.2×10^6/well). The compound was diluted from 30 μg/mL to 3 ng/mL in assay buffer (final conc. 10 μg/mL to 1 ng/mL). Each 40 μL of cells, compound, and Eu-labeled MAdCAM-1 chimeric protein at 3 nM (final conc. at 1 nM) was gently mixed in a round-bottom 96-well plate (Costar no. 3799) for 1 h at room temperature. The mixture was transferred into a 96-well silent screen plate (Nunc) that had been previously blocked with 1% BSA in HSM (25 mM HEPES, 150 mM NaCl, 2 mM $MgCl_2$) for 1 h at room temperature. The free ligands were removed by centrifugation of the plate at 2000 rpm for 1 min at 4 °C. The plate was washed with wash buffer (25 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM $MnCl_2$) three times. Enhancement solution (100 μL) was added to each well. After gentle shaking for 5 min, the solution was transferred to an enzyme immunoassay (EIA) plate (Costar no. 3590), and the time-resolved fluorescence (TRF) of each well was measured.

mLFA-1/Eu-Human ICAM-1 Binding Assay. Membrane-bound human LFA-1 (mLFA-1) was immobilized on EIA/RIA 96-well plates (Corning 3590, Corning) by adding 40 μL of 1 μg/mL mLFA-1 dissolved in 0.1% OG (*N*-octyl β-D-glucopyranoside)−HSM (25 mM HEPES(pH7.5), 140 mM NaCl, 2 mM $MgCl_2$) to each well and incubating overnight at 4 °C. After removal of the nonadherent mLFA-1, each well was blocked with 200 μL/well of 1% BSA/HSM for 1 h at room temperature. The plates were washed three times with 200 μL/well of chilled HSM, and 50 μL/well of 2 nM Eu-labeled human ICAM-1 D1D5-IgG prepared in dilution buffer (0.01% BSA, 20 μM DTPA−HSM) was added, then allowed to bind to the immobilized mLFA-1 for 2 h at room temperature. The inhibitory activity of the compounds was assessed by adding the compound to the Eu-ICAM-1 preparation with the DMSO concentration of 5%. The plates were washed three times with 200 μL/well of chilled HSM, and 150 μL of the DELFIA enhancement solution (PerkinElmer Inc.) was added to each well. The Eu-fluorescence was measured using a time-resolved fluorometer (DELFIA Research fluorometer, model 1234-001, PerkinElmer Inc.) after gentle shaking for 5 min.

$\alpha_{IIb}\beta_3$-Dependent Platelet Aggregation Assay. Blood was collected from two in-house volunteers using syringes with a 1/10 volume of 3.8% sodium citrate (International Reagents Corp.) as an anticoagulant. The blood was centrifuged at

1500 × *g* for 10 min at room temperature, and the platelet-rich plasma (PRP) supernatant was separated. The residue was further centrifuged at 2500 × *g* for 15 min, and platelet-poor plasma (PPP) supernatant was obtained. Two-hundred microliters of PRP and 1 μL of compound prepared in DMSO were added to a cuvette and incubated at 37 °C for 2 min, and platelet agglutination was induced by adding 2 μL of 10 μg/mL collagen (Collagenreagent HORM, Nycomed). For 10 min after agglutination induction, the platelet agglutination rate was measured with a measuring device (HEMA TRACER 313, MC medical).

Estimated Serum Concentration. Female BALB/c mice (8 weeks old, Charles River Japan, Inc.) were used. Each group consisted of four animals. The mice were orally administered the compound dissolved in 0.5% (w/v) methylcellulose (MC) at a dose of 10 (mg/mL)/kg. After 15 min, blood samples were collected via the inferior vena cava from the animals under ether anesthesia. The blood samples were left to stand at room temperature and centrifuged at 2000 rpm for 10 min at 4 °C. The serum samples were subsequently stored in a −20 °C freezer prior to analysis. According to the VLA-4/VCAM-1 binding assay, instead of the compound solution, 50 μL of serum samples at various concentrations were added into each well (final concentration 0.01−10%). As for the calibration curve, each diluted compound solution was also assayed in the presence of the same concentration of untreated mouse serum.

Murine Asthma Model. Female BALB/c AnNCrj mice (8 weeks old, Charles River Japan, Inc.) received an oral administration of cyclophosphamide dissolved in water at a dose of 150 mg/kg (day 0). On day 2 and 14, 500 μg of protein of *Ascaris suum* extract (LSL Co., Ltd.) in 0.2 mL of saline-containing 4.5 mg of aluminum hydroxide was injected intraperitoneally. On day 22, the mice were challenged intratracheally under anesthesia with 300 μg (30 μL) of protein of *Ascaris suum* extract. In the negative control group, sensitized mice were challenged with saline instead of the antigen.

Effect on Eosinophil Infiltration. Test compounds dissolved in 0.5% MC containing 0.03% Tween 80 were orally administered 15 min before and 8, 24, and 32 h after the antigen challenge at a dose of 1.67, 5, or 15 mg/kg (for **13e**−**f**, **14e**) and 5, 15, or 45 mg/kg (for **14d**). Forty-eight hours after the antigen challenge, the mice were sacrificed, and BAL fluid was collected using tracheal polyethylene cannula with 2 × 0.5 mL of Hanks' balanced salt solution. The cells in the BAL fluid were counted with a particle analyzer, CDA-500 (Sysmex Corp.). Cytocentrifuged preparations (Cytospin 2; Shandon) were stained with Wright's stain solution (Muto Chemical Co., Ltd.) for differential counts, based on standard morphologic criteria. The number of eosinophils was calculated by multiplying the total cell number by the percentage of eosinophils in the cytocentrifuged preparations.

Effect on Hyper-responsiveness. Compound **14e**, which was dissolved in 0.5% MC containing 0.03% Tween 80, was orally administered 15 min before and 8, 24, and 32 h after the antigen challenge at a dose of 2 or 12.5 mg/kg. The bronchial hyperresponsiveness in each mouse was estimated from the increase in lung resistance by acetylcholine chloride (ACh; Sigma Corp.) injection at 48 h after the antigen challenge. Ten minutes before the start of the measurement, the mice were anesthetized by an intraperitoneal injection of pentobarbital at a dose of 100 mg/kg. The trachea was cannulated and connected to a rodent ventilator (MiniVent type 845; Hugo Sachs Electronik-Harvard Apparatus) with an in-line pressure transducer (TRD-4510; Buxco Electronics, Inc.) that was coupled to a pulmonary mechanics analyzer (Bio-System XA; Buxco Electronics, Inc.). The flows were determined by measuring the differential pressure (TRD-5100; Buxco Electronics, Inc.) across eight layers of 400-mesh wire cloth covering a 1.3-cm hole in a plethysmograph box (Plyan-M; Buxco Electorics, Inc.). The mice were placed in the plethysmogragh box and then ventilated at 140 strokes/min with a stroke volume of 150 μL. After establishing a stable baseline of lung resistance, ACh, dissolved in saline was cumulatively administered (25, 50, 100, and 200 (μg/mL)/kg) via the tail vein, and the changes in lung resistance were monitered.

Guinea Pig Asthma Model. Using an ultrasonic nebulizer (NE-U12; OMRON Corp.) and a vinyl chloride box (W 300 × H 390 × D 570 mm^3), male guinea pigs (Kud; Hartley (Kudo Ltd.), $n = 8$/group) were exposed to an aerosol mist of physiological saline solution containing 1% ovalbumin (OVA) for 10 min/day, for 8 consecutive days. At 1 week after the final sensitization, each animal was restrained in a Pulmos chamber (W 115 × H 140 × D 410 mm^3), and with an ultrasonic nebulizer, the animals were exposed to an aerosol mist of physiological saline solution containing 2% OVA for 5 min. At 24 and 1 h before the OVA challenge, metyrapone (10 (mg/mL)/kg) was intravenously administered, and at 30 min before the OVA challenge, pyrilamine (10 (mg/mL)/kg) was intraperitoneally administered. At about 22−27.5 h after the antigen challenge, each guinea pig was restrained in the Pulmos chamber (W 115 × H 140 × D 410 mm^3), and with the ultrasonic nebulizer, physiological saline solution and acetylcholine chloride (ACh) solution at 0.0625, 0.125, 0.25, 0.5, 1, and 2 mg/mL were stepwise introduced as aerosol mist, allowing each dose to be inhaled for 1 min. Using an integrated respiratory function analysis system (Pulmos-I, M.I.P.S. Co.), the measurement of sRaw was continued until it reached 2-fold the baseline sRaw value (sRaw following inhalation of physiological saline solution). From the concentration of ACh and the sRaw concentration vs resistance curve, the concentration of ACh necessary to elevate sRaw 100% over the baseline sRaw (PC_{100}ACh) was calculated using CA-Cricket Graph III 1.5.2 software. Compound **14e** was orally administered (0.8, 2, 5, or 12.5 mg/kg) at 1 h before the antigen challenge, prior to the treatment by metyrapone, and at 2.5 h after the challenge in each group. Dexamethasone was orally administered at 16 and 2 h before the antigen challenge.

Pharmacokinetic Studies on Rats. Male Sprague−Dawley rats (7 weeks old, SLC Japan) were used. The animals were fasted for 18 h prior to dosing. Each group consisted of four animals. The rats were orally administered compounds at the dose of 1 mg/kg dissolved in 0.5% (w/v) MC with 3 equiv of NaOH aqueous solution. The rats were intravenously administered compounds at a dose of 1 mg/kg dissolved in saline with 3 equiv of NaOH solution. Blood samples (0.4 mL) were collected at 0.08 (or 0.25 for po), 0.5, 1, 2, and 6 h after the administration. These analytical samples were left to stand at room temperature, followed by centrifugation at 15000 rpm for 10 min at 4 °C. The plasma fractions were subsequently stored in a −20 °C freezer until analyzed. The concentrations of the compounds were determined by an LC/MS/MS method, comprised of an Alliance 2695 HPLC (Waters), Symmetry Shield RP8, i.d. 2.1 mm × 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 time data were analyzed by noncompartmental approaches using the WinNonlin software program (version 1.13.1, Pharsight, Mountain View, CA).

Pharmacokinetic Studies on Dogs. Male beagle dogs (10−12 kg, LSG Corp.) were used. The animals were fasted for 18 h prior to dosing. Each group consisted of three animals. The test compounds were dissolved in 0.5% (w/v) MC with 3 equiv of NaOH for oral cassette dosing or dissolved in saline with 3 equiv of NaOH for intravenous cassette dosing. The dose in each experiment was 0.5 mg/kg. Blood samples (1 mL) were collected after 0.08 (for iv), 0.25 (for po), 0.5, 1, 2, 4, 8, and 24 h. After the 4 h sampling, the animals were provided with food. These analytical samples were prepared and analyzed according to the pharmacokinetic studies on rats.

Pharmacokinetic Studies on Monkeys. Female cynomolgus monkeys (3.5−4 kg, HAMRI Co.) were used. The animals were

fasted for 18 h prior to dosing. Each group consisted of three animals. Compound **14e** was suspended in 0.5% (w/v) MC for oral dosing (0.5 mg/kg) or dissolved in saline with 3 equiv of NaOH for intravenous dosing (0.5 mg/kg). Blood samples (1 mL) were collected after 0.08 (for iv), 0.25 (for po), 0.5, 1, 2, 4, 8, and 24 h. After the 4 h sampling, the animals were provided with food. These analytical samples were prepared and analyzed according to the pharmacokinetic studies on rats.

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