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Tricyclic pharmacophore-based molecules as novel integrin $\alpha_v\beta_3$ antagonists. Part III: Synthesis of potent antagonists with $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual activity and improved water solubility

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Abstract—In order to optimize our novel integrin $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists, spatial screening at the N-terminus was performed. The $\alpha_v\beta_3$ antagonistic activity varied depending on the space that was occupied by the N-terminus, but high potency against $\alpha_{IIb}\beta_3$ was well maintained. The (3*S*)-aminopiperidine analogue had the strongest activity against $\alpha_v\beta_3$, and the *S* isomer at piperidine was more potent than the *R* isomer. Compounds selected on the basis of SAR analysis of a novel lead compound showed acceptable early absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles and sufficient water solubility for use as infusion drugs. Docking studies with the $\alpha_v\beta_3$ receptor were performed to confirm the SAR findings.

1. Introduction

The vitronectin receptor integrin $\alpha_v\beta_3$ binds to a number of proteins, including vitronectin, fibrinogen, and osteopontin, through recognition of the tripeptide RGD sequence.[1] Orally active, small-molecular antagonists of integrin $\alpha_v\beta_3$[2] are expected to have utility in the treatment of several chronic diseases, including osteoporosis, cancer, diabetic retinopathy, rheumatoid arthritis, and restenosis. We are interested in integrin $\alpha_v\beta_3$ antagonists as injectable drugs, because $\alpha_v\beta_3$ is involved in adhesion and migration of vascular smooth muscle cells and leukocytes.[3] Fab fragment of the human-murine monoclonal antibody Abciximab,[4] which binds to the $\alpha_v\beta_3$ receptor and $\alpha_{IIb}\beta_3$ receptor, is already used to treat ischemic diseases. Therefore, injectable $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists are likely to be of therapeutic value in the treatment of acute ischemic diseases. The preceding paper of this report enclosed the discovery of highly constrained $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists,[5] which had significant inhibitory effects in several ischemia/reperfusion models compared to a selective $\alpha_v\beta_3$ antagonist or a selective $\alpha_{IIb}\beta_3$ antagonist.[6] Subsequently, we explored another type of $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonist with potent activity and good water solubility.

Spatial screening by using cyclic peptides, as proposed by Kessler et al.,[7] is one possible approach to enhance the $\alpha_v\beta_3$ antagonistic activity of lead compounds. They studied the optimum conformation of the RGD sequence and the optimum distance from the N-terminus to the C-terminus for $\alpha_v\beta_3$ antagonistic activity by synthesis and analysis of many kinds of cyclic peptides with rigid RGD conformations. In the case of non-peptide $\alpha_v\beta_3$ antagonists, several groups have analyzed the optimum length for $\alpha_v\beta_3$-antagonistic activity by modifying the length of linear molecules.[8] However, it was difficult to determine the relationship between the optimum distance from the N-terminus to the C-terminus and the activity, because the three-dimensional structure of the $\alpha_v\beta_3$ receptor[9] was not known at that time. Moreover, so-called linear molecules could have many stable conformations. We thought that spatial screening of our prototype $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonist **1**[5a] might be useful, because the conformation of **1** is highly constrained, and spatial optimization of the N-terminus might be possible by changing the 4-aminopiperidine moiety.

Keywords: Integrin $\alpha_v\beta_3$ antagonist; Integrin $\alpha_{IIb}\beta_3$ antagonist; Acute ischemic disease; 3-aminopiperidine derivatives.
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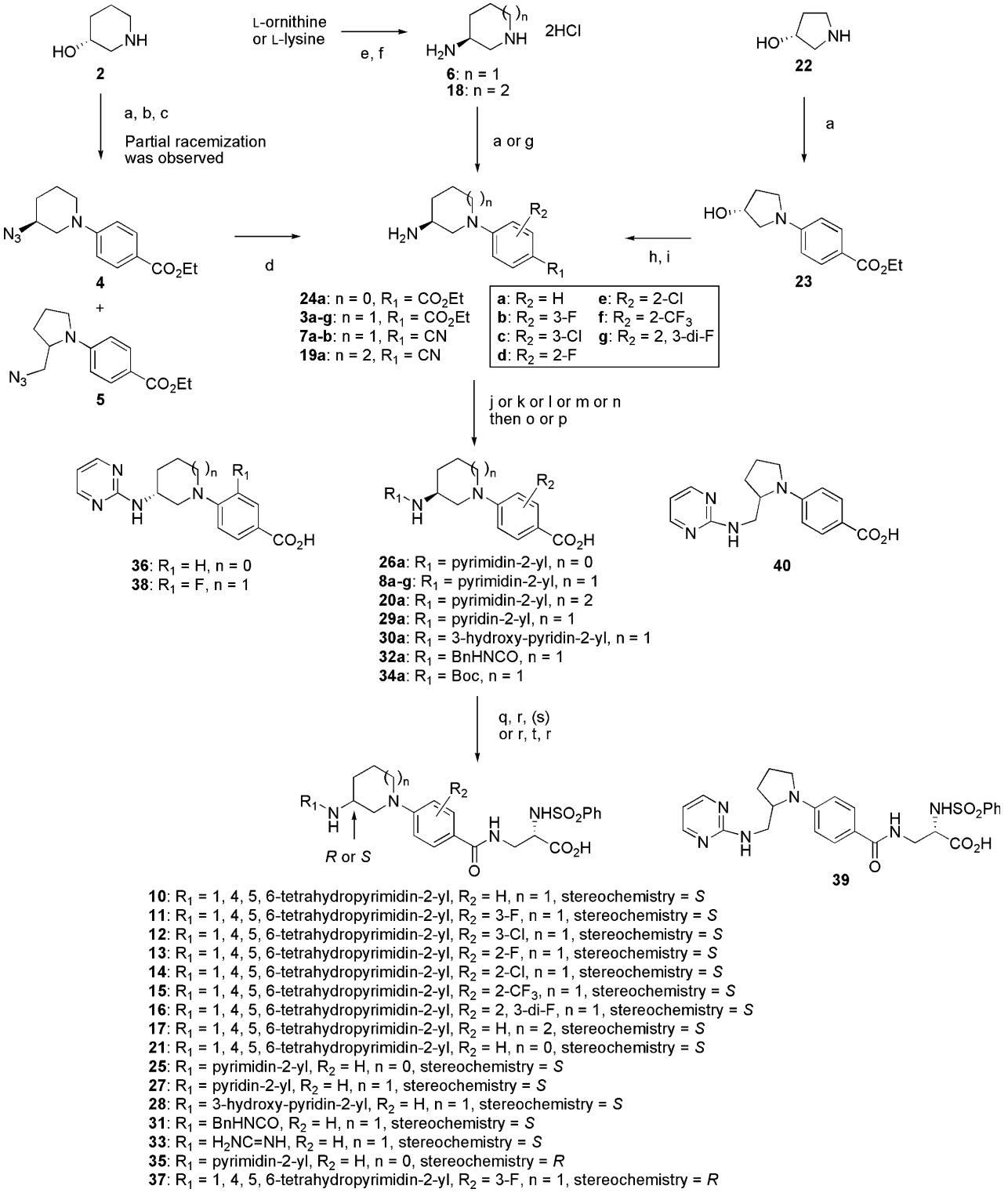
2. Chemistry

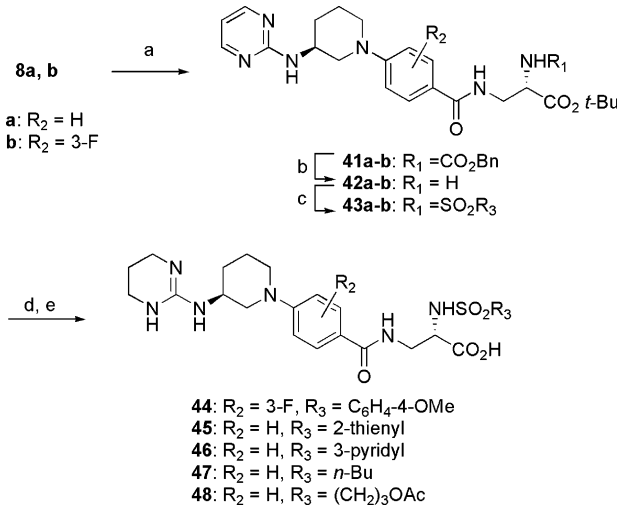
Scheme 1 shows the synthetic scheme for novel compounds for detailed spatial optimization of the N-terminus by modifying 4-aminopiperidine. Initially, (3*R*)-hydroxypiperidine (**2**) was selected as a starting material for compound **3a**. After nucleophilic substitution[10] with ethyl 4-fluorobenzoate, conversion of alcohol to azide



Scheme 1. Reagents: (a) ethyl 4-fluorobenzoate, DMSO or NMP; (b) MsCl, Et_3N, CH_2Cl_2; (c) NaN_3, DMF; (d) Pd/C, H_2, dioxane; (e) $SOCl_2$, MeOH then IRA-400; (f) $LiAlH_4$, THF; (g) 4-fluorobenzonitrile, $NaHCO_3$, NMP; (h) phthalimide, ADDP, *n*-Bu_3P, benzene; (i) hydrazine, MeOH; (j) 2-bromopyrimidine, *i*-Pr_2EtN, DMSO or NMP, (k) 2-chloropyridine, $Pd(OAc)_2$, BINAP, NaO*t*-Bu, toluene; (l) 6-methoxy-2-chloropyridine, $Pd(OAc)_2$, BINAP, NaO*t*-Bu, toluene; (m) benzyl isocyanate, CH_3CN; (n) Boc_2O, Et_3N, DMF; (o) NaOH, THF, MeOH, H_2O; (p) H_2SO_4; (q) *t*-butyl (2*S*)−*N*-benzenesulfonyl-2,3-diaminopropionate (**9a**), BOP, *i*-Pr_2EtN, DMF; (r) TFA, CH_2Cl_2; (s) 10% Pd/C, H_2, dioxane, H_2O; (t) 1*H*-pyrazole-1-carboxamidine hydrochloride *i*-Pr_2EtN, dioxane, H_2O.

via the mesylate gave the desired azidopiperidine (**4**) with an azidomethylpyrrolidine (**5**) as a by-product.[11] Because optical rotation data indicated that partial racemization had occurred in the amine (**3a**) derived from **4**, an alternative route was required. Selective nucleophilic substitution of 4-fluorobenzoate with a secondary amine, (3*S*)-aminopiperidine hydrochloride (**6**),[12] derived from L-ornithine gave the desired compound **3a**. In addition, the reaction of **6** with 4-fluorobenzonitrile afforded **7a**. When the benzonitrile was used, a higher yield was obtained than in the case of the benzoate, possibly owing to the strong electron-withdrawing effect of the nitrile group. After introduction of a pyrimidine at the amino group of the ester (**3a**) or nitrile (**7a**), basic or acidic hydrolysis gave a carboxylic acid (**8a**). The optical purity of **8a** (>95%) was determined by chiral HPLC. The carboxylic acid (**8a**) was coupled with a diaminopropionate (**9a**)[13] to afford an amide. Removal of the *t*-butyl group using TFA, followed by hydrogenolysis of the pyrimidine ring, finally gave the desired molecule, **10**. Compounds **11**, **12**, **13**, **14**, **15**, and **16** were analogously prepared via **8b–g** from substituted benzoate. The synthetic route for **10** was applied to the preparation of the (3*S*)-aminoazepane analogue (**17**). (3*S*)-Aminoazepane hydrochloride (**18**) derived from L-lysine was converted to an amine (**19a**), a carboxylic acid (**20a**), and then **17**. Next, the (3*S*)-aminopyrrolidine analogue (**21**) was successfully obtained by application of the established method. Thus, nucleophilic substitution of 4-fluorobenzoate with (3*R*)-hydroxypyrrolidine (**22**) afforded compound **23**. This was converted to an amine (**24a**), and **25** was synthesized via amidation of the carboxylic acid **26a** with the amine **9a**, and acid hydrolysis. Hydrogenolysis of the pyrimidine ring gave **21**. The N-terminal pyridine analogues **27** and **28** were produced by palladium-catalyzed coupling reactions[14] of chloropyridine analogues with **3a** and **7a**, respectively. A benzylurea derivative, **31**, was synthesized via **32a** that was obtained from **3a** and benzyl isocyanate. For synthesis of the guanidine **33**, the N-terminal functionality was introduced at the final stage of the synthetic route. After *N*-Boc protection of the amine **3a**, hydrolysis of the ester afforded **34a**. Amidation of **34a** with **9a** furnished an amide. After selective deprotection of the Boc group using TFA at 4 °C, the guanidine (**33**) was finally prepared by reaction with a pyrazole reagent[15] and then TFA at room temperature. Analogously to the synthetic route to **25**, the *R* derivative (**35**) was obtained via a carboxylic acid (**36**) derived from *ent*-**23** that was synthesized by Mitsunobu inversion[16] of the alcohol (**23**). Similarly, the *R* derivative **37** was synthesized via a carboxylic acid (**38**) that was derived from D-ornithine as a starting material. An aminomethylpyrrolidine analogue (**39**) was also synthesized from the by-product (**5**) via the carboxylic acid **40**.

Compounds modified at the C-terminus were synthesized through a convergent route[5b] as shown in Scheme 2. After coupling reactions of carboxylic acids (**8a–b**) and an amine (**9b**),[5a] the Cbz group of compounds **41a–b** was selectively reduced to give the amines **42a–b**. After coupling of the amine **42a** or **42b** with each sulfonyl chloride, removal of the *t*-butyl group and then hydrogenolysis of the pyrimidine ring afforded **44**, **45**, **46**, **47**, and **48**.



Scheme 2. Reagents: (a) *t*−butyl (2*S*)-*N*-benzyloxycarbonyl-2-3-diaminopropionate (**9b**), BOP, *i*-Pr_2EtN, DMF; (b) Pd/C, H_2, THF; (c) R_3SO_2Cl, *i*-Pr_2EtN, DMF; (d) TFA, CH_2Cl_2; (e) Pd/C, H_2, dioxane, H_2O.

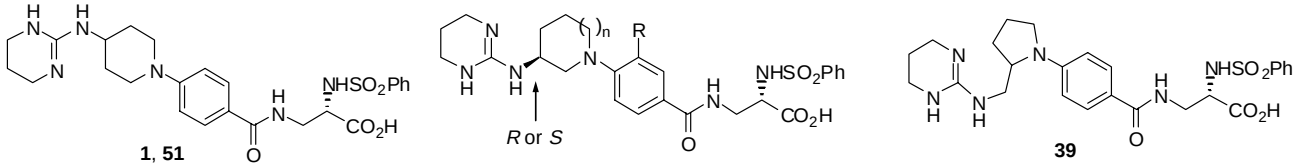
3. Results and discussion

All novel compounds were evaluated in $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ receptor binding assays as an initial screening. Compounds that exhibited strong inhibition were picked up and further evaluated in $\alpha_v\beta_3$-mediated cell adhesion assays using VSMC (human vascular smooth muscle cell) and human vitronectin. Because these compounds are zwitterionic,[5b] the solubility of the free form in 10% aq DMSO was preliminarily examined. Solubility in water was evaluated quantitatively for several promising compounds.

3.1. Structure–activity relationships

The results of spatial optimization of the N-terminus are summarized in Table 1. Interestingly, the (3*S*)-aminopyrrolidine analogue (**25**) showed $\alpha_v\beta_3$-antagonistic activity stronger than that of its *R* isomer (**35**). However, such a difference was not observed in the case of $\alpha_{IIb}\beta_3$-antagonistic activity. This result encouraged us to synthesize *S* derivatives for further structure–activity relationships (SAR) analysis. Next, the $\alpha_v\beta_3$-antagonistic activities of the 4-aminopiperidine analogue (**1**), (3*S*)-aminopyrrolidine analogue (**21**), (3*S*)-aminopiperidine analogue (**10**), (3*S*)-aminoazepane analogue (**17**), and 2-(aminomethyl)pyrrolidine analogue (**39**) were compared for optimization of the space at the N-terminus. Compound **10** showed the strongest activity against $\alpha_v\beta_3$. Although the $\alpha_v\beta_3$-antagonistic activities varied depending on the space that the N-terminus occupied, every molecule was a potent $\alpha_{IIb}\beta_3$ antagonist. The reason for these results is not clear, but ligand recognition by the $\alpha_v\beta_3$ receptor might be more restrictive than that by the $\alpha_{IIb}\beta_3$ receptor. Furthermore, **10** showed strong

Table 1. Spatial Screening of the N-Terminus



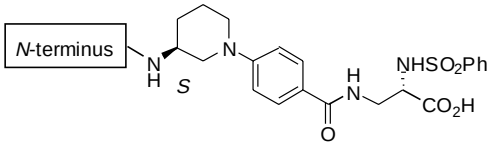
Compound	*n*	Stereochemistry	R	IC_{50} (nM)			Solubility[a]
				$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC	
1	—	—	—	1.3	3.1	190	<1.0
51[b,c]	—	—	—	120	8.1	n.t.	n.t.
25[b]	0	*S*	H	110	1.6	n.t.	n.t.
35[b]	0	*R*	H	1,600	1.3	n.t.	n.t.
21	0	*S*	H	320	3.0	n.t.	n.t.
10	1	*S*	H	0.48	0.56	31	>2.0
17	2	*S*	H	6.0	0.47	n.t.	n.t.
39	—	—	—	5.4	0.46	400	>2.0
11	1	*S*	F	0.13	1.1	180	>2.0
37	1	*R*	F	49	0.26	n.t.	n.t.

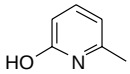
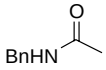
[a] Maximum concentration as the free form in 10% aq DMSO (mg/ml).
[b] Pyrimidine at the N-terminus.
[c] See Ref. 5b.

inhibitory activity against cell adhesion and good solubility. It was confirmed that the *S* isomer (**11**) showed activity stronger than that of the *R* isomer (**37**) in the case of 3-aminopiperidine analogues. So, we chose the (3*S*)-aminopiperidine analogue (**10**) as a novel lead compound and decided to modify it further to obtain more precise SAR data.

The SAR at the N-terminus is summarized in Table 2. We found that a cyclic guanidine analogue structure was favorable for receptor binding inhibition, cell adhesion inhibition, and solubility.[5b] Thus, we examined several guanidine mimetics that are known to confer good affinity for $\alpha_v\beta_3$. A pyridine analogue[2b,8e,17] (**27**) showed potent activity at sub-nanomolar concentration with good solubility. On the other hand, a guanidine analogue (**33**) and a benzylurea[18] (**31**) showed weaker activity. In order to improve the water solubility of **27**, a hydroxyl group was introduced at the pyridine moiety to furnish **28**, but surprisingly, this compound had no activity. The reason for this is not clear, but **28** might exist in a pyridone form under the assay conditions.

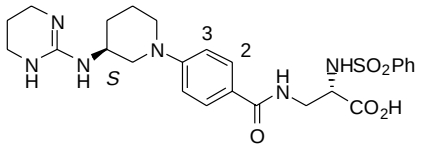
Table 2. Structure–activity relationships at the N-terminus



Compound	N-terminus	IC_{50} (nM)			Solubility[a]
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC	
10		0.48	0.56	31	>2.0
33		1.5	0.29	n.t.	n.t.
27		0.76	0.73	350	>2.0
28		>100	0.63	>1,000	n.t.
31		36	0.93	n.t.	n.t.

[a] Maximum concentration as the free form in 10% aq DMSO (mg/ml).

Table 3. Structure–activity relationships at the central aromatic ring



Compound	2 and/or 3 Position	IC_{50}(nM)			Solubility[a]
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC	
10	unsubstituted	0.48	0.56	31	>2.0
11	3-F	0.13	1.1	180	>2.0
12	3-Cl	3.2	0.34	1,300	>2.0
13	2-F	0.23	0.78	16	>2.0
14	2-Cl	1.1	2.4	130	n.t.
15	2-CF_3	1.9	1.8	290	n.t.
16	2,3-di-F	0.40	1.0	71	>2.0

[a] Maximum concentration as the free form in 10% aq DMSO (mg/ml).

Finally, the results in the cell adhesion inhibition assay led us to select tetrahydropyrimidine as the N-terminus.

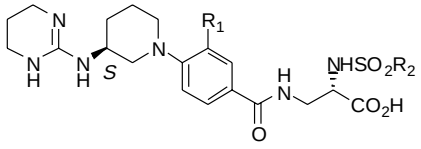
The SAR at the central aromatic ring is summarized in Table 3. Mono-fluorinated derivatives **11** and **13** showed more potent receptor binding inhibition than **10** and possessed good solubility. Whereas **11** showed decreased potency in the cell adhesion assay, **13** showed strong inhibition of cell adhesion. Introduction of a chlorine atom at the 3-position (**12**) decreased the $\alpha_v\beta_3$-antagonistic activity, although the activity against $\alpha_{IIb}\beta_3$ was maintained. Introduction of a chlorine atom (**14**) or a trifluoromethyl group (**15**) at the 2-position also decreased the $\alpha_v\beta_3$-antagonistic activity. These SAR were different from those of the piperazine[5a] or 4-aminopiperidine[5b] derivatives, whose activities were enhanced when a substituent was introduced into the aromatic ring.

The SAR at the α substituent is summarized in Table 4. First, we decided to substitute at the benzene ring, because arylsulfonamides had shown the strongest activity in the preceding paper of this report.[5b] A methoxyl group (**44**) or a hydroxyl group (**49**) was introduced, but the results were disappointing. Then, the benzene ring itself was replaced. In the case of the thiophene analogue (**45**), the activity and the solubility were maintained at the same levels as in the case of **10**. However, the pyridine analogue (**46**) or *n*-butyl derivative (**47**) showed reduced activity. Compound **50**, in which a hydrophilic functionality was introduced into the alkylsulfonamide to improve the solubility, and its precursor (**48**) also showed reduced activity.

3.2. Early absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles

After SAR analysis, we selected compounds **10**, **13**, and **45**, which exhibit both strong inhibition of $\alpha_v\beta_3$-mediated cell adhesion and good solubility (>2 mg/ml), and their quantitative water solubility, pharmacokinetics in rats, acute toxicity in mice, and mutagenic activity were evaluated. All compounds showed satisfactory water solubility and pharmacokinetic parameters for intravenous infusion (Table 5). In particular, the water solubility of these three compounds was markedly

Table 4. Structure–activity relationships at the sulfonamide moiety



Compound	R_1	R_2	IC_{50} (nM)			Solubility[a]
			$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC	
10	H	Ph	0.48	0.56	31	>2.0
44	F	C_6H_4-4-OMe	5.3	2.7	n.t.	n.t.
49	F	C_6H_4-4-OH	3.7	7.3	n.t.	n.t.
45	H	2-Thienyl	0.25	0.40	52	>2.0
46	H	3-Pyridinyl	1.3	0.90	n.t.	n.t.
47	H	*n*-Bu	1.3	1.3	n.t.	n.t.
48	H	$(CH_2)_3OAc$	1.1	0.72	n.t.	>2.0
50	H	$(CH_2)_3OH$	1.7	1.7	150	>2.0

[a] Maximum concentration as the free form in 10% aq DMSO (mg/ml).

Table 5. Quantitative water solubility and rat pharmacokinetics[a] of selected compounds

Compound	Water solubility (mg/ml)	$t_{1/2}$(min)	CL (ml/min/kg)	AUC (ng min/ml)	V_{ss} (ml/kg)
1	<0.1				
10	3.5	36	63	8320	856
13	2.8	27	63	7925	536
45	3.3	27	62	8110	719

[a] Dosage of 0.5 mg/kg, iv.

improved compared to that of **1**. Furthermore, none of the compounds had significant toxicity.[19] These data indicated that the $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists **10**, **13** and **45** are potential candidate drugs for treatment of reperfusion injury.

3.3. Docking studies

Recently, Xiong et al. have reported the X-ray structure analysis of the $\alpha_v\beta_3$ receptor complex with a cyclic peptide.[9b] In order to confirm the results of SAR studies of our highly constrained $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists, **1**, **10**, and **21** were docked into the three-dimensional structure of the $\alpha_v\beta_3$ receptor. As shown in Figure 1A, **1** and **10** were suggested to bind to the receptor in a way similar to the cyclic peptide. The predicted major interactions were hydrogen bonding of NH at the N-terminus and Asp218 in the α_v chain, coordination of the carboxyl group at the C-terminus and the Mn^{2+} ion of MIDAS (the metal ion-dependent adhesion site), hydrogen bonding from the sulfonamide NH to the carbonyl group of the main chain at Tyr122 in the β_3 chain, stacking of the aromatic ring of the sulfonamide and the aromatic ring of Tyr122, and hydrogen bonding of the carbonyl group at the central amide bond and Arg214 in the β_3 chain.

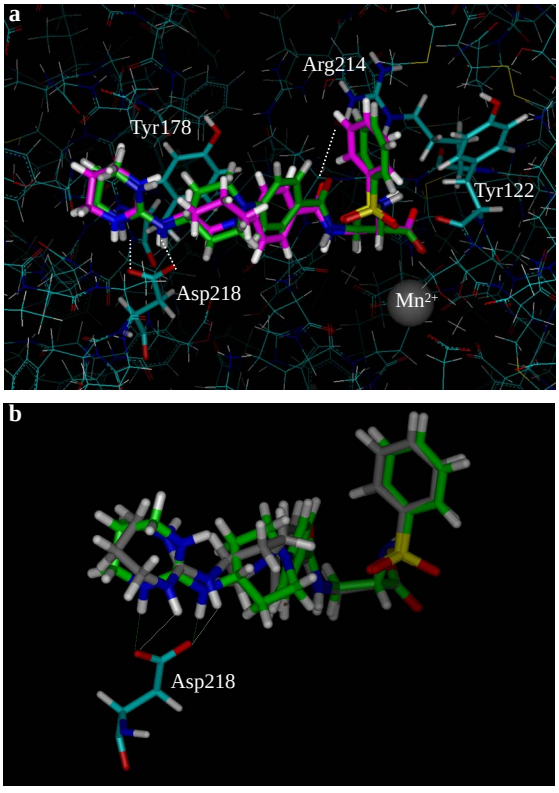


Figure 1. Molecular models of **1** (green), **10** (magenta) and **21** (gray) in the binding site of integrin $\alpha_v\beta_3$. (a) Representative interactions with amino acids of $\alpha_v\beta_3$. (b) Geometry of hydrogen bonds to Asp218.

When the 4-aminopiperidine analogue (**1**) was compared with the 3-aminopiperidine analogue (**10**) to confirm the results of spatial screening, the N-termini of the two molecules surprisingly appeared to occupy almost the same space. On the other hand, the piperidine rings appeared to occupy different spaces. The piperidine ring of **1** might have an unfavorable steric interaction with Tyr178 in the α_v chain due to its torsion angle with the central aromatic ring. This steric hindrance might reduce the activity of **1** as compared with that of **10**. Next, when the docking mode of **21** was compared with that of **10** or **1**, it appeared that the N-terminus was bound differently to the receptor, as shown in Figure 1B. Compound **1**, possessing strong activity, might appear to interact with Asp218 in the α_v chain more strongly than does **21**, because the geometry of the hydrogen bonds of **1** might be more favorable.[20]

Other results indicated that there is little space around the amide bond; substitution at the amide bond led to decreased activity.[5a] Finally, these docking data support the SAR finding that the acidity of the sulfonamide is important for the activity,[5b] because the acidity is related to the polarization of sulfonamide NH, which is an important factor for the interaction with Tyr122 in the β_3 chain.

4. Conclusion

In summary, our novel (3*S*)-aminopiperidine-based $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists appear to be good candidates for use as injectable drugs. For optimization, spatial screening at the N-terminus was performed by modifying 4-aminopiperidine of the prototype $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonist, **1**. The strongest activity against $\alpha_v\beta_3$ was observed in (3*S*)-aminopiperidine derivatives. The *S* stereocenter of piperidine derivatives imparted stronger antagonistic activity against $\alpha_v\beta_3$ than the *R* center, and the $\alpha_v\beta_3$ receptor might recognize ligand molecules in a more restricted manner compared to the $\alpha_{IIb}\beta_3$ receptor. Docking studies on the $\alpha_v\beta_3$ receptor were consistent with the SAR. Recently, similar results in spatial screening of small molecules that possess $\alpha_v\beta_3$ antagonistic activity were reported.[21] Although the new lead compound **10** possesses two asymmetric centers, both of them can be easily synthesized from general L-amino acids at reasonable cost. We selected

10, **13**, and **45** on the basis of SAR analysis, and they showed stronger cell adhesion inhibition and better water solubility than our selected antagonists in the preceding paper of this report.[5b] Furthermore, preliminary ADMET profiles of these compounds indicated that they might be suitable for use as infusion drugs. Like our selected antagonists in the preceding paper of this report, these compounds may have utility in the treatment of acute ischemic diseases.

5. Experimental section

^{1}H NMR spectra were recorded on JNM-LA400 spectrometers with chemical shifts reported in ppm with internal tetramethylsilane as a basis. Electron ionization (EI) mass spectra were recorded on a Hitachi M-80B instrument. Fast-atom bombardment (FAB) mass spectra were recorded on a JEOL JMS-700 instrument. Thermospray (TSP) mass spectra were recorded on a Hewlett-Packard 5989A instrument. Electrospray ionization (ESI) mass spectra were recorded on a Hewlett-Packard 5989A instrument. Atmospheric pressure chemical ionization (APCI) mass spectra were recorded on a Hewlett-Packard 5989A instrument. High-resolution mass spectra (HRMS) were recorded under FAB conditions. Optical rotations were obtained on a JASCO DIP-370 polarimeter.

5.1. Ethyl 4-{(3*S*)-azidopiperidin-1-yl}benzoate (4) and ethyl 4-{2-(azidomethyl)pyrrolidin-1-yl}benzoate (5)

DMSO (10 ml) was added to (3*R*)-hydroxypiperidine (**2**) (1.00 g, 9.90 mmol), to which ethyl 4-fluorobenzoate (1.45 ml, 9.90 mmol) was added. The mixture was stirred at 100 °C for 29 h and left until it returned to room temperature. The reaction mixture was then poured into aqueous $NaHCO_3$ (200 ml), and the title compound was extracted three times with AcOEt. The organic layers were combined and washed twice with saturated brine (100 ml). It was then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to give ethyl 4-{(3*R*)-hydroxypiperidin-1-yl}benzoate (778 mg, 29%) as a light brown syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CH_2CH_3), 1.65 (1H, m, piperidine), 1.92 (3H, m, piperidine), 3.15 (2H, m, piperidine), 3.37 (1H, m, piperidine), 3.57 (1H, dd, piperidine), 3.91 (1H, m, piperidine), 4.33 (2H, q, CH_2CH_3), 6.89 (2H, d, C_6H_4), 7.92 (2H, d, C_6H_4); EIMS *m*/*z* 249 (M^+).

CH_2Cl_2 (27 ml) was added to the alcohol (727 mg, 2.65 mmol), to which Et_3N (1.03 ml, 7.42 mmol) was added. Methanesulfonyl chloride (287 μl, 3.71 mmol) was gradually added dropwise at room temperature, and the mixture was stirred at that temperature for 30 min. Aqueous $NaHCO_3$ (1 L) was added to stop the reaction, and the mixture was extracted three times with AcOEt (500 ml). The organic layers were combined and dried over anhydrous Na_2SO_4. It was then concentrated under reduced pressure to give ethyl 4-{(3*R*)-(methanesulfonyloxy)-piperidin-1-yl}benzoate as a brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CH_2CH_3), 1.69 (1H, m, piperidine), 1.93 (2H, m, piperidine), 2.07 (1H, m, piperidine), 3.03 (3H, s, Ms), 3.21 (1H, ddd, piperidine), 3.40 (1H, dd, piperidine), 3.46 (1H, m, piperidine), 3.77 (1H, dd, piperidine), 4.33 (2H, q, CH_2CH_3), 4.85 (1H, dddd, piperidine), 6.89 (2H, d, C_6H_4), 7.93 (2H, d, C_6H_4).

DMF (9.0 ml) was added to the crude compound, to which sodium azide (207 mg, 3.18 mmol) was added. The mixture was stirred at 80 °C for 3 h and left until it returned to room temperature. The reaction solution was then poured into water (100 ml), and the title compound was extracted three times with AcOEt (200 ml). The organic layers were combined and washed twice with water (200 ml). It was then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give an about 1:1 mixture (704 mg, 97% in two steps) of **5** with **4** as a brown syrup; EIMS *m*/*z* 274 (M^+).

5.2. Ethyl 4-{(3*S*)-aminopiperidin-1-yl}benzoate (3a) from 4

1,4-Dioxane (23.0 ml) was added to the about 1:1 mixture (624 mg, 2.27 mmol) of **5** with **4**. To the solution 10% palladium on charcoal (120 mg) was added. The mixture was vigorously stirred under a hydrogen pressure of 1 atm at room temperature for 6 h. Insolubles were filtered, and washed twice with 1,4-dioxane and twice with MeOH. The filtrate and the washings were combined and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 7:1) to give **3a** (149 mg, 26%) as a brown syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (1H, m, piperidine), 1.36 (3H, t, CH_2CH_3), 1.66 (1H, m, piperidine), 1.82 (1H, m, piperidine), 1.98 (1H, dddd, piperidine), 2.70 (1H, dd, piperidine), 2.94 (2H, m, piperidine), 3.63 (1H, ddd, piperidine), 3.74 (1H, dddd, piperidine), 4.33 (2H, q, CH_2CH_3), 6.87 (2H, d, C_6H_4), 7.91 (2H, d, C_6H_4); TSPMS *m*/*z* 249 $(M+H)^+$; $[\alpha]_D^{22}$ −13° (*c* 0.50, MeOH).

And ethyl 4-{2-(aminomethyl)pyrrolidin-1-yl}benzoate was also obtained as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CH_2CH_3), 2.05 (4H, m, pyrrolidine), 2.67 (1H, dd, NH_2CH_2), 2.92 (1H, dd, NH_2CH_2), 3.25 (1H, m, pyrrolidine), 3.50 (1H, m, pyrrolidine), 3.81 (1H, m, pyrrolidine), 4.33 (2H, q, CH_2CH_3), 6.58 (2H, d, C_6H_4), 7.90 (2H, d, C_6H_4), TSPMS *m*/*z* 249 $(M+H)^+$.

5.3. (3*S*)-Aminopiperidine (6)

MeOH (120 ml) was added to L-ornithine hydrochloride (20.0 g, 119 mmol), and the suspension was cooled to −78 °C. Thionyl chloride (25.7 ml, 297 mmol) was added dropwise to the cooled suspension in the internal temperature range of −78 °C to −45 °C over a period of 20 min. Fifteen minutes after the completion of the dropwise addition, the temperature of the mixture was raised to room temperature, and the mixture was further vigorously stirred for 13 h. The reaction solution was concentrated under reduced pressure, and the residue

was further dried by a vacuum pump for 3 h. The obtained amorphous was purified with an Amberlite IRA-400 (OH^-) anion exchange resin (130 g) to give (3*S*)-aminopiperidin-2-one as a crude product. A mixed solution composed of $CHCl_3$ (800 ml) and MeOH (80 ml) was added to the crude compound. The insolubles were filtered through Celite. A mixed solvent composed of $CHCl_3$ (400 ml) and MeOH (40 ml) was added again to 9.6 g of the insolubles, and the insolubles were then filtered. The filtrates were combined followed by concentration to give (3*S*)-aminopiperidin-2-one (13.7 g, 77%) as a colorless solid: ^{1}H NMR (400 MHz, D_2O) δ 1.48 (1H, m, piperidine), 1.72 (2H, m, piperidine), 1.99 (1H, dddd, piperidine), 3.16 (2H, dd, piperidine), 3.24 (1H, dd, piperidine); EIMS *m*/*z* 114 (M^+).

THF (270 ml) was added to lithium aluminum hydride (2.52 g, 66.4 mmol). (3*S*)-Aminopiperidin-2-one (4.0 g, 26.6 mmol) was gradually added to the cooled suspension in the internal temperature range of 5 °C to 16 °C. Ten minutes after the completion of the addition, the mixture was warmed to room temperature and further vigorously stirred for 3 h. Lithium aluminum hydride (202 mg, 5.32 mmol) was added to the mixture, and the mixture was stirred for 50 min. The mixture was ice cooled, and water (2.7 ml), 5.0 M aqueous NaOH (2.7 ml), and water (9.1 ml) were added to the cooled mixture. The mixture was warmed to room temperature and vigorously stirred for 1.5 h. The precipitated inorganic material was filtered through Celite and was then washed with THF. The filtrate and the washings were combined and dried over anhydrous Na_2SO_4. 4.0 M aqueous HCl in AcOEt (13.3 ml, 53.2 mmol) was added to the mixture, and the solvent was removed by distillation under reduced pressure. The residue was subjected to azeotropic distillation with MeOH to give hydrochloride of **6** (3.57 g, 78%) as a brown solid: ^{1}H NMR (400 MHz, CD_3OD) (as hydrochloride) δ 1.75 (1H, dddd, piperidine), 1.90 (1H, m, piperidine), 2.09 (1H, ddddd, piperidine), 2.23 (1H, br d, piperidine), 3.02 (1H, ddd, piperidine), 3.09 (1H, dd, piperidine), 3.41 (1H, br d, piperidine), 3.62 (2H, m, piperidine); EIMS *m*/*z* 100 (M^+).

5.4. Ethyl 4-{(3*S*)-aminopiperidin-1-yl}benzoate (3a) from 6

N-Methyl-2-pyrrolidone (2.0 ml) was added to **6** (200 mg, 1.16 mmol). $NaHCO_3$ (487 mg, 5.80 mmol) and ethyl 4-fluorobenzoate (85 μl, 0.580 mmol) were added to the solution. The mixture was stirred at 120 °C for 23 h. Water (50 ml) and saturated brine (50 ml) were then added to the reaction solution, and the mixture was extracted three times with AcOEt. The combined organic layers were washed with a mixed solution composed of saturated brine (50 ml) and water (50 ml), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 7:1) to give **3a** (61.2 mg, 42%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (1H, m, piperidine), 1.36 (3H, t, CH_2CH_3), 1.63 (1H, m, piperidine), 1.82 (1H, m, piperidine), 1.98 (1H, m, piperidine), 2.74 (1H, dd, piperidine), 2.95 (2H, m, piperidine), 3.58 (1H, dt, piperidine), 3.71 (1H, m, piperidine), 4.32 (2H, q, CH_2CH_3), 6.86 (2H, d, C_6H_4), 7.89 (2H, d, C_6H_4); TSPMS *m*/*z* 249 $(M+H)^+$; $[\alpha]_D^{22}$ 49° (*c* 0.71, MeOH).

5.5. 4-{(3*S*)-Aminopiperidin-1-yl}benzonitrile (7a)

$NaHCO_3$ (21.4 g, 255 mmol) was suspended in *N*-methyl-2-pyrrolidone (30 ml) in a sealed tube. 4-Fluorobenzonitrile (6.7 g, 55.5 mmol) and **6** (14.3 g, 82.6 mmol) were added to the sealed tube. The mixture was stirred at room temperature for 5 min in such a state that the system was opened. Thereafter, the system was hermetically sealed, and, in this state, stirring was carried out at 120 °C for 22 h. The reaction solution was cooled to room temperature, and the reaction mixture was then purified by silica gel column chromatography (CH_2Cl_2/MeOH/conc. NH_4OH = 100:10:1) to give **7a** (9.89 g, 89%) as a light yellow syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (1H, dddd, piperidine), 1.63 (1H, ddddd, piperidine), 1.79–1.87 (1H, m, piperidine), 1.95–2.04 (1H, m, piperidine), 2.74 (1H, dd, piperidine), 2.90–2.99 (2H, m, piperidine), 3.61 (1H, ddd, piperidine), 3.72 (1H, dddd, piperidine), 6.86 (2H, d, C_6H_4), 7.47 (2H, d, C_6H_4); TSPMS *m*/*z* 202 $(M+H)^+$.

5.6. 4-{(3*S*)-(Pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (8a) from 3a

N-Methylpyrrolidone (0.40 ml) was added to **3a** (50 mg, 0.20 mmol). *i*-Pr_2EtN (0.18 ml, 1.0 mmol) and 2-bromopyrimidine (35 mg, 0.24 mmol) were added to the solution, and the mixture was stirred at 110 °C for 20 h. Water (50 ml) was then added to the reaction solution, and the mixture was extracted three times with AcOEt. The combined organic layers were dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 7:3) to give ethyl 4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (38 mg, 58%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (3H, t, CH_2CH_3), 1.69 (1H, m, piperidine), 1.74 (1H, m, piperidine), 1.85 (1H, m, piperidine), 2.02 (1H, m, piperidine), 3.06 (1H, dd, piperidine), 3.40 (1H, ddd, piperidine), 3.50 (1H, m, piperidine), 3.84 (1H, dd, piperidine), 4.14 (1H, m, piperidine), 4.32 (2H, q, CH_2CH_3), 5.24 (1H, d, NH), 6.55 (1H, t, pyrimidine), 6.91 (2H, d, C_6H_4), 7.89 (2H, d, C_6H_4), 8.28 (2H, d, pyrimidine), TSPMS *m*/*z* 327 $(M+H)^+$; $[\alpha]_D^{25}$ 34° (*c* 0.35, MeOH).

To a solution of this benzoate (156 mg, 0.480 mmol) in a mixture of THF (7.2 ml) and MeOH (2.4 ml), 1 M aqueous NaOH (2.4 ml) was added. The reaction mixture was stirred for 6 h at 40 °C and concentrated. The residue was added H_2O (30 ml) and washed with AcOEt (30 ml). The aqueous layer was adjusted to pH 4 by the addition of 1.0 M aqueous HCl. The precipitate was collected by a glass filter, washed with water, and then dried to give **8a** (110 mg, 77%) as a colorless solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.52–1.61 (2H, m, piperidine), 1.73–1.81 (1H, m, piperidine), 1.93–2.01 (1H, m, piperidine), 2.76 (1H, dd, piperidine), 2.87 (1H, ddd, piperidine), 3.79–3.91 (2H, m, piperidine), 3.98 (1H, br d, piperidine), 6.58 (1H, t, pyrimidine),

6.96 (2H, d, C_6H_4), 7.74 (2H, d, C_6H_4), 8.29 (2H, d, pyrimidine); EIMS *m*/*z* 298 (M^+).

Optical purity of **8a** was evaluated with a chiral HPLC. HPLC analyses were performed according to the following conditions. Column, CHIRALCEL OD (Daicel Chemical Industries, LTD) 250 mm × 4.6 mm; eluent, hexane/2-propanol/diethylamine/TFA = 70:30:0.1:0.1; flow rate, 1.0 ml/min; wavelength, 254 nm; temp, 25 °C; injection volume, 10 μl (1 mg/ml EtOH solution). The retention time of **8a** was 10.96 min, whereas the retention time of its enantiomer was 8.64 min.

5.7. 4-{(3*S*)-(Pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (8a) from 7a

DMSO (250 ml) was added to 2-bromopyrimidine (8.72 g, 54.9 mmol) and **7a** (9.89 g, 49.2 mmol). *i*-Pr_2EtN (50 ml, 287 mmol) was added to the solution, and the mixture was vigorously stirred at 120 °C for 12 h. The reaction solution was cooled to room temperature. Water (500 ml) was then added to the reaction solution, and the mixture was extracted three times with AcOEt (500 ml). The combined organic layers were washed three times with water (500 ml) and once with saturated brine (500 ml), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1 hen only AcOEt) to give 4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzonitrile (10.5 g, 77%) as a light yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60–1.72 (1H, m, piperidine), 1.75 (1H, dddd, piperidine), 1.83–1.92 (1H, m, piperidine), 2.01–2.10 (1H, m, piperidine), 3.03 (1H, dd, piperidine), 3.17 (1H, ddd, piperidine), 3.56 (1H, ddd, piperidine), 3.92 (1H, dd, piperidine), 4.09 (1H, ddddd, piperidine), 6.58 (1H, t, pyrimidine), 6.92 (2H, d, C_6H_4), 7.47 (2H, d, C_6H_4), 8.30 (2H, d, pyrimidine); EIMS *m*/*z* 279 (M^+).

The above nitrile (7.75 g, 27.7 mmol) was dissolved in 50% H_2SO_4 (28 ml), and the solution was then heated under reflux for 2 h. The reaction solution was cooled to room temperature, and the reaction solution was then slowly poured into aqueous $NaHCO_3$ (800 ml) under ice cooling. The mixture was adjusted to pH 4 by the addition of 1.0 M aqueous HCl. The precipitate was collected by a glass filter, washed with water, and then dried to give **8a** (8.11 g, 98%) as a colorless solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.52–1.61 (2H, m, piperidine), 1.73–1.81 (1H, m, piperidine), 1.93–2.01 (1H, m, piperidine), 2.76 (1H, dd, piperidine), 2.87 (1H, ddd, piperidine), 3.79–3.91 (2H, m, piperidine), 3.98 (1H, br d, piperidine), 6.58 (1H, t, pyrimidine), 6.96 (2H, d, C_6H_4), 7.74 (2H, d, C_6H_4), 8.29 (2H, d, pyrimidine); EIMS *m*/*z* 298 $(M)^+$.

5.8. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (10)

(Step 1) DMF (3.4 ml) was added to **8a** (50 mg, 0.17 mmol). *i*-Pr_2EtN (0.087 ml, 0.50 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) (90 mg, 0.20 mmol) were added to the solution, and the mixture was stirred at room temperature for 10 min. *t*-Butyl (2*S*)-*N*-benzenesulfonyl-2,3-diaminopropionate (**9a**)[13] (60 mg, 0.20 mmol) was then added, and the mixture was stirred at room temperature for 14 h. Water was added to the reaction solution, and the mixture was extracted three times with AcOEt. The combined organic layers were dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9:1) to give *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (93 mg, 95%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (9H, s, *t*-Bu), 1.66 (1H, m, piperidine), 1.75 (1H, m, piperidine), 1.86 (1H, m, piperidine), 1.99 (1H, m, piperidine), 3.02 (1H, dd, piperidine), 3.16 (1H, ddd, piperidine), 3.48 (1H, m, piperidine), 3.58 (1H, ddd, CONH*CH*$_2$CH), 3.81 (1H, dd, piperidine), 3.89 (2H, m, CONH*CH*$_2$*CH*), 4.15 (1H, m, piperidine), 5.30 (1H, d, NH), 5.78 (1H, d, NH), 6.52 (1H, m, NH), 6.55 (1H, t, pyrimidine), 6.93 (2H, d, C_6H_4), 7.47 (2H, m, C_6H_5), 7.55 (1H, m, C_6H_5), 7.66 (2H, d, C_6H_4), 7.84 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 581 $(M+H)^+$; $[\alpha]_D^{25}$ 75° (*c* 0.26, $CHCl_3$).

(Step 2) CH_2Cl_2 (1.3 ml) was added to the above ester (72 mg, 0.13 mmol). Trifluoroacetic acid (1.3 ml) was added to the solution, and the mixture was stirred at room temperature for 5 h. The reaction solution was concentrated under reduced pressure to give a trifluoroacetate of (2*S*)-benzenesulfonylamino-3-[4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid as a colorless solid.

(Step 3) 1,4-Dioxane (2.0 ml) and water (0.2 ml) were added to the trifluoroacetate of the crude compound (0.072 mmol). To the solution 10% palladium on charcoal (13 mg) was added, and the mixture was vigorously stirred under a hydrogen pressure of 1 atm at room temperature for 3 h. The reaction solution was filtered through Celite and washed with 1,4-dioxane and water. The filtrate and the washings were combined, and the combined solution was concentrated under reduced pressure. The residue was purified by preparative thin-layer silica gel column chromatography (CH_2Cl_2/EtOH/water/conc. NH_4OH = 8:8:1:1) and was then purified by Sephadex LH-20 (MeOH) to give **10** (26 mg, 68%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.62 (1H, m, piperidine), 1.75 (1H, m, piperidine), 1.87 (1H, m, piperidine), 1.98 (3H, m, piperidine and tetrahydropyrimidine), 3.12 (1 H, dd, *J* = 7.3 Hz, 12.7 Hz, piperidine), 3.18 (1 H, m, piperidine), 3.33 (1 H, m, piperidine), 3.36 (4 H, t, *J* = 5.6 Hz, tetrahydropyrimidine), 3.54 (2 H, m, CONH*CH*$_2$CH and piperidine), 3.66 (2H, m, CONH*CH*$_2$CH and piperidine), 3.87 (1H, dd, *J* = 5.1 Hz, 8.3 Hz, CONHCH$_2$*CH*), 6.97 (2H, d, *J* = 8.9 Hz, C_6H_4), 7.46 (2H, m, C_6H_5), 7.53 (1H, m, C_6H_5), 7.69 (2H, d, *J* = 8.9 Hz, C_6H_4), 7.84 (2H, m, C_6H_5); ^{13}C NMR (DMSO-d_6) δ 19.7, 22.8, 30.2, 37.8, 42.6, 46.3, 47.3, 52.4, 55.6, 114.2, 123.7, 126.6,

128.1, 129.0, 132.2, 140.8, 152.2, 152.4, 165.5, 172.9; TSPMS *m/z* 529 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_5S$: 529.2233, found: 529.2236; $[\alpha]_D^{28}$ 54° (*c* 0.23, MeOH).

Compounds **3b**–**3f** were prepared using the procedures described for preparing **3a** from **6**.

5.9. Methyl 4-{(3*S*)-aminopiperidin-1-yl}-3-fluorobenzoate (3b)

Methyl 3,4-difluorobenzoate (1.29 g, 7.5 mmol) and **6** (2.6 g, 15.0 mmol) afforded **3b** (0.55 g, 29%) as a brown syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (1H, m, piperidine), 1.73 (1H, m, piperidine), 1.86 (1H, m, piperidine), 1.96 (1H, m, piperidine), 2.66 (1H, dd, piperidine), 2.85 (1H, m, piperidine), 3.04 (1H, m, piperidine), 3.35 (1H, m, piperidine), 3.46 (1H, m, piperidine), 3.87 (3H, s, OCH_3), 6.90 (1H, dd, C_6H_3), 7.64 (1H, dd, C_6H_3), 7.72 (1H, dd, C_6H_3); FABMS 253 $(M+H)^+$.

5.10. Ethyl 4-{(3*S*)-aminopiperidin-1-yl}-2-fluorobenzoate (3d)

Ethyl 2,4-difluorobenzoate (230 mg, 1.2 mmol) and **6** (630 mg, 3.7 mmol) afforded **3d** (84 mg, 26%) as a brown syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (1H, m, piperidine), 1.36 (3H, t, CH_2CH_3), 1.61 (1H, m, piperidine), 1.81 (1H, m, piperidine), 1.93 (1H, m, piperidine), 2.71 (1H, dd, piperidine), 2.92 (2H, m, piperidine), 3.60 (1H, dt, piperidine), 3.71 (1H, m, piperidine), 4.32 (2H, q, CH_2CH_3), 6.49 (1H, dd, C_6H_3), 6.61 (1H, dd, C_6H_3), 7.78 (1H, dd, C_6H_3); TSPMS *m/z* 267$(M+H)^+$.

5.11. Ethyl 4-{(3*S*)-aminopiperidin-1-yl}-2-chlorobenzoate (3e)

Ethyl 2-chloro-4-fluorobenzoate (200 mg, 1.0 mmol) and **6** (346 mg, 2.0 mmol) afforded **3e** (113 mg, 40%) as a brown syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (1H, m, piperidine), 1.37 (3H, t, CH_2CH_3), 1.63 (1H, m, piperidine), 1.81 (1H, m, piperidine), 1.99 (1H, m, piperidine), 2.69 (1H, dd, piperidine), 2.92 (2H, m, piperidine), 3.60 (1H, m, piperidine), 3.70 (1H, m, piperidine), 4.33 (2H, q, CH_2CH_3), 6.72 (1H, dd, C_6H_3), 6.86 (1H, d, C_6H_3), 7.80 (1H, d, C_6H_3); EIMS *m/z* 282 (M^+).

5.12. Ethyl 4-{(3*S*)-aminopiperidin-1-yl}-2-trifluoromethylbenzoate (3f)

Ethyl 4-fluoro-2-trifluoromethylbenzoate (750 mg, 3.2 mmol) and **6** (1.1 g, 6.4 mmol) afforded **3f** (579 mg, 58%) as a brown syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (1H, m, piperidine), 1.36 (3H, t, CH_2CH_3), 1.64 (1H, m, piperidine), 1.84 (1H, m, piperidine), 2.02 (1H, m, piperidine), 2.73 (1H, dd, piperidine), 2.95 (2H, m, piperidine), 3.62 (1H, m, piperidine), 3.73 (1H, m, piperidine), 4.33 (2H, q, CH_2CH_3), 6.93 (1H, dd, C_6H_3), 7.13 (1H, d, C_6H_3), 7.79 (1H, d, C_6H_3); EIMS *m/z* 316 (M^+).

5.13. 3-Fluoro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (8b)

The title compound **8b** (54.6 mg, 15% in two steps) was synthesized from **3b** (550 mg, 2.1 mmol) as a colorless solid following the general procedure for **8a** from **3a**; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.50 (1H, m, piperidine), 1.66 (1H, m, piperidine), 1.82 (1H, m, piperidine), 1.94 (1H, m, piperidine), 2.63 (1H, m, piperidine), 2.73 (1H, br t, piperidine), 3.30 (1H, m, piperidine), 3.56 (1H, br d, piperidine), 3.99 (1H, m, piperidine), 6.57 (1H, t, pyrimidine), 7.05 (1H, t, C_6H_3), 7.49 (1H, dd, C_6H_3), 7.61 (1H, dd, C_6H_3), 8.28 (2H, d, pyrimidine), TSPMS *m/z* 317 $(M+H)^+$.

5.14. 3-Chloro-4-{(3*S*)-(pyrimidin-2-ylamino)-piperidin-1-yl}benzoic acid (8c)

N-Methylpyrrolidone (40 ml) was added **6** (2.5 g, 15 mmol), and $NaHCO_3$ (4.0 g, 48 mmol) and methyl 3-chloro-4-fluorobenzoate (1.4 g, 7.5 mmol) were added to the solution. The mixture was stirred at 110 °C for 15 h to afford **3c**. *i*-Pr_2EtN (10.4 ml, 60 mmol) and 2-bromopyrimidine (3.6 g, 22 mmol) were then added to the reaction solution, and the mixture was stirred at 100 °C for 20 h. Water (50 ml) was then added to the mixture, and the mixture was extracted three times with AcOEt. The combined organic layers were dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to give methyl 3-chloro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (370 mg, 14%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 (2H, m, piperidine), 1.87 (1H, m, piperidine), 1.97 (1H, m, piperidine), 3.11 (3H, m, piperidine), 3.32 (1H, m, piperidine), 3.88 (3H, s, OMe), 4.30 (1H, m, piperidine), 5.69 (1H, brs, NH), 6.51 (1H, t, pyrimidine), 7.04 (1H, d, C_6H_3), 7.86 (1H, dd, C_6H_3), 8.01 (1H, d, C_6H_3), 8.26 (2H, d, pyrimidine), TSPMS *m/z* 347 $(M+H)^+$.

THF (13.5 ml) and MeOH (4.5 ml) were added to the above benzoate (310 mg, 0.90 mmol), and 1 M aqueous NaOH (4.5 ml) was added to the solution. The mixture was stirred at 40 °C for 4 h and then concentrated under reduced pressure. Water (20 ml) was added to the residue, and the solution was then washed twice with AcOEt. The aqueous layer was adjusted to pH 4 by the addition of 1 M aqueous HCl. The precipitated solid was separated by centrifugation and then dried to give **8c** (210 mg, 70%) as a colorless solid; FABMS *m/z* 333 $(M+H)^+$.

The following compounds were prepared using the procedures described for preparing **8a** from **3a**.

5.15. 2-Fluoro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (8d)

Ester **3d** (144 mg, 0.54 mmol) afforded **8d** (45 mg, 28% in two steps) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.77 (2H, m, piperidine), 1.92 (1H, m, piperidine), 2.09 (1H, m, piperidine), 3.16 (2H, m, piperidine), 3.69 (1H, m, piperidine), 3.92 (1H, m, piperidine), 4.17

(1H, m, piperidine), 6.71 (1H, dd, C_6H_3), 6.79 (1H, dd, C_6H_3), 6.99 (1H, t, pyrimidine), 7.78 (1H, dd, C_6H_3), 8.58 (2H, m, pyrimidine).

5.16. 2-Chloro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (8e)

Ester **3e** (100 mg, 0.35 mmol) afforded **8e** (32 mg, 40% in two steps) as a colorless solid; EIMS *m/z* 332 (M^+).

5.17. 4-{(3*S*)-(Pyrimidin-2-ylamino)piperidin-1-yl}-2-trifluoromethylbenzoic acid (8f)

Ester **3f** (200 mg, 0.63 mmol) afforded **8f** (90 mg, 44% in two steps) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 (2H, m, piperidine), 1.94 (1H, m, piperidine), 2.11 (1H, m, piperidine), 3.23 (2H, m, piperidine), 3.72 (1H, m, piperidine), 3.94 (1H, m, piperidine), 4.20 (1H, m, piperidine), 7.02 (1H, t, pyrimidine), 7.18 (1H, dd, C_6H_3), 7.33 (1H, d, C_6H_3), 7.85 (1H, d, C_6H_3), 8.50 (2H, br s, pyrimidine).

5.18. 2,3-Difluoro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (8g)

Methyl 2,3,4-trifluorobenzoate (1.43 g, 7.52 mmol) and **6** (15.0 mmol) afforded methyl 2,3-difluoro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (0.17 g, 7.0%) as a colorless syrup according to the procedure for **8c**; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75 (2H, m, piperidine), 1.94 (2H, m, piperidine), 3.07 (1H, dd, piperidine), 3.16 (1H, ddd, piperidine), 3.32 (1H, m piperidine), 3.58 (1H, dd, piperidine), 3.89 (3H, s, OCH_3), 4.21 (1H, m, piperidine), 5.38 (1H, d, NH), 6.54 (1H, t, pyrimidine), 6.75 (1H, ddd, C_6H_2), 7.60 (1H, ddd, C_6H_2), 8.27 (2H, d, pyrimidine); EIMS *m/z* 348 (M^+).

Hydrolysis of the above ester afforded **8g** (104 mg, 72%) as a colorless solid; EIMS *m/z* 334 (M^+).

Compounds **11**–**16** were prepared using the procedures described for preparing **10** from **8a**.

5.19. (2*S*)-Benzenesulfonylamino-3-[3-fluoro-4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (11)

Compound **8b** (54.6 mg, 0.173 mmol) afforded **11** (80.0 mg, 85% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.66 (1H, m, piperidine), 1.78 (1H, m, piperidine), 1.90 (2H, m, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 3.12 (3H, m, piperidine), 3.25 (1H, dd, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.53 (1H, dd, CONHC*H*$_2$CH), 3.66 (1H, dd, CONHC*H*$_2$CH), 3.72 (1H, m, piperidine), 3.75 (1H, dd, CONHCH$_2$C*H*), 7.06 (1H, t, C_6H_3), 7.51 (4H, m, C_6H_5 and C_6H_3), 7.58 (1H, dd, C_6H_3), 7.86 (2H, m, C_6H_5); TSPMS *m/z* 547 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}N_6O_5FS$: 547.2139, found: 547.2148; $[\alpha]_D^{26}$ 87° (*c* 1.0, MeOH).

5.20. (2*S*)-Benzenesulfonylamino-3-[3-chloro-4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (12)

Compound **8c** (90.0 mg, 0.27 mmol) afforded **12** (22.0 mg, 78% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.64 (1H, m, piperidine), 1.83 (1H, m, piperidine), 1.96 (4H, m, piperidine and tetrahydropyrimidine), 3.12 (1H, m, piperidine), 3.20 (2H, m, piperidine), 3.36 (4H, t, tetrahydropyrimidine), 3.49 (2H, m, CONH*CH*$_2$CH and piperidine), 3.70 (2H, m, CONH*CH*$_2$CH and piperidine), 3.98 (1H, dd, CONHCH$_2$*CH*), 7.16 (1H, d, C_6H_3), 7.46 (3H, m, C_6H_5), 7.69 (1H, dd, C_6H_3), 7.81(3H, m, C_6H_5 and C_6H_3); TSPMS *m/z* 563 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}N_6O_5ClS$: 563.1843, found: 563.1830; $[\alpha]_D^{28}$ 51° (*c* 0.17, MeOH).

5.21. (2*S*)-Benzenesulfonylamino-3-[2-fluoro-4-{(3S)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (13)

Compound **8d** (45.0 mg, 0.14 mmol) afforded **13** (6.0 mg, 10% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.63 (1H, m, piperidine), 1.70 (1H, m, piperidine), 1.86 (1H, m, piperidine), 1.97 (3H, m, piperidine and tetrahydropyrimidine), 3.12 (1H, m, piperidine), 3.20 (1H, m, piperidine), 3.36 (4H, t, tetrahydropyrimidine), 3.42 (1H, m, piperidine), 3.65 (4H, m, CONH*CH*$_2$CH and piperidine), 3.85 (1H, m, CONHCH$_2$*CH*), 6.67 (1H, dd, J = 1.4 Hz, 9.0 Hz, C_6H_3), 6.80 (1H, dd, J = 1.4 Hz, 15.8 Hz, C_6H_3), 7.46 (2H, m, C_6H_5), 7.50 (1H, m, C_6H_5), 7.72 (1H, dd, J = 9.0 Hz, 9.0 Hz, C_6H_3), 7.83 (2H, m, C_6H_5); ^{13}C NMR (DMSO-d_6) δ 19.7, 22.8, 30.2, 37.8, 42.8, 46.4, 46.6, 51.6, 55.1, 100.6 (d, J_{CF} = 28.1 Hz), 109.9 (d, J_{CF} = 12.4 Hz), 110.3, 126.6, 129.0, 131.6 (d, J_{CF} = 5.0 Hz), 132.4, 140.5, 152.2, 153.8 (d, J_{CF} = 10.8 Hz), 161.0 (d, J_{CF} = 219.2 Hz), 162.2, 162.4, 173.1; TSPMS *m/z* 547 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}N_6O_5FS$: 547.2139, found: 547.2148; $[\alpha]_D^{28}$ 35° (*c* 0.17, MeOH).

5.22. (2*S*)-Benzenesulfonylamino-3-[2-chloro-4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (14)

Compound **8e** (30.0 mg, 0.09 mmol) afforded **14** (8.0 mg, 33% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.64 (1H, m, piperidine), 1.75 (1H, m, piperidine), 1.88 (2H, m, piperidine), 1.97 (2H, m, tetrahydropyrimidine), 3.13 (3H, m, piperidine), 3.18 (1H, m, piperidine), 3.36 (4H, t, tetrahydropyrimidine), 3.50 (2H, m, CONH*CH*$_2$CH), 3.75 (2H, m, CONHCH$_2$*CH* and piperidine), 6.90 (1H, dd, C_6H_3), 6.96 (1H, d, C_6H_3), 7.51 (4H, m, C_6H_5 and C_6H_3), 7.85 (2H, d, C_6H_5); TSPMS *m/z* 563 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}N_6O_5ClS$: 563.1843, found: 563.1830; $[\alpha]_D^{26}$ 12° (*c* 0.086, MeOH).

5.23. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}-2-trifluoromethylbenzoylamino]propionic acid (15)

Compound **8f** (80.0 mg, 0.22 mmol) afforded **15** (10.0 mg, 19% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.63 (1H, m, piperidine), 1.74 (1H, m, piperidine), 1.90 (2H, m, piperidine), 1.96 (2H, m, tetrahydropyrimidine), 3.19 (3H, m, piperidine), 3.29 (1H, m, piperidine), 3.36 (4H, t, tetrahydropyrimidine), 3.58 (3H, m, CONHC*H*$_2$CH and piperidine), 3.72 (1H, dd, CONHCH$_2$C*H*), 7.17 (1H, dd, C_6H_3), 7.19 (1H, d, C_6H_3), 7.53 (3H, m, C_6H_5), 7.60 (1H, d, C_6H_3), 7.86 (1H, d, C_6H_5); TSPMS *m*/*z* 597 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{26}H_{31}N_6O_5F_3S$: 597.2107, found: 597.2092; $[\alpha]_D^{26}$ 50° (*c* 0.12, MeOH).

5.24. (2*S*)-Benzenesulfonylamino-3-[2,3-difluoro-4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (16)

Compound **8g** (50.0 mg, 0.15 mmol) afforded **16** (35.0 mg, 91% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.67 (1H, m, piperidine), 1.78 (1H, m, piperidine), 1.91 (2H, m, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 3.12 (3H, m, piperidine), 3.19 (1H, m, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.63 (3H, m, CONHC*H*$_2$CH and piperidine), 3.76 (1H, dd, CONHCH$_2$C*H*), 6.85 (1H, dd, C_6H_2), 7.50 (4H, m, C_6H_2 and C_6H_5), 7.84 (2H, m, C_6H_5); TSPMS *m*/*z* 565 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{30}N_6O_5F_2S$: 565.2045, found: 565.2053; $[\alpha]_D^{28}$ 39° (*c* 0.55, MeOH).

5.25. (3*S*)-Aminoazepane (18)

L-α-Amino-ε-caprolactam hydrochloride (Fluka) (4.5 g, 27.3 mmol) afforded **18** (4.60 g, 90%) as a brown solid according to **6** from L-ornithine hydrochloride; ^{1}H NMR (400 MHz, CD_3OD) (as hydrochloride) δ 1.72 (1H, m, azepane), 1.93 (4H, m, azepane), 2.21 (1H, m, azepane), 3.30 (2H, m, azepane), 3.61 (2H, m, azepane), 3.73 (1H, dddd, azepane); EIMS *m*/*z* 114 (M^+).

5.26. 4-{(3*S*)-Aminoazepan-1-yl}benzonitrile (19a)

The title compound **19a** (210.6 mg, 37%) was synthesized from **18** (1.0 g, 5.34 mmol) and 4-fluorobenzonitrile (323 mg, 2.67 mmol) as a colorless syrup following the general procedure for **7a**; ^{1}H NMR (400 MHz, CD_3OD) δ 1.29 (1H, m, azepane), 1.44 (1H, m, azepane), 1.78 (3H, m, azepane), 2.02 (1H, m, azepane), 3.06 (2H, m, azepane), 3.35 (1H, dd, azepane), 3.74 (1H, ddd, azepane), 3.84 (1H, br d, azepane), 6.83 (2H, br d, C_6H_4), 7.46 (2H, br d, C_6H_4).

5.27. 4-{(3*S*)-(Pyrimidin-2-ylamino)azepan-1-yl}benzoic acid (20a)

Compound **19a** (100 mg, 0.464 mmol) afforded **20a** (95.9 mg, 67%) as a brown solid according to **8a** from **7a**; TSPMS *m*/*z* 313 $(M+H)^+$.

5.28. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)azepan-1-yl}benzoylamino]propionic acid (17)

Compound **20a** (50.0 mg, 0.160 mmol) afforded **17** (49.1 mg, 57% in three steps) as a colorless solid according to **10** from **8a**; ^{1}H NMR (400 MHz, CD_3OD) δ 1.57 (2H, m, azepane), 1.75 (3H, m, azepane), 1.95 (1H, m, azepane), 1.97 (2H, quintet, tetrahydropyrimidine), 3.37 (4H, dd, tetrahydropyrimidine), 3.39 (1H, m, azepane), 3.56 (1H, dd, CONHC*H*$_2$CH), 3.70 (5H, m, azepane and CONHC*H*$_2$C*H*), 3.84 (1H, m, azepane), 6.75 (2H, br d, C_6H_4), 7.50 (2H, m, C_6H_5), 7.56 (1H, m, C_6H_5), 7.70 (2H, br d, C_6H_4), 7.87 (2H, m, C_6H_5); FAB-MS *m*/*z* 543 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{26}H_{34}N_6O_5S$: 543.2390, found: 543.2404; $[\alpha]_D^{26}$ 100° (*c* 0.55, MeOH/conc. NH_4OH = 10:1).

5.29. Ethyl 4-{(3*R*)-hydroxypyrroridin-1-yl}benzoate (23)

To a solution of (*R*)-(+)-pyrroridinol (**22**) (3.50 g, 40.2 mmol) in DMSO (20 ml) ethyl 4-fluorobenzoate (8.8 ml, 60.3 mmol) was added and the reaction mixture was stirred at 110 °C for 2 days. Saturated aqueous NH_4Cl (300 ml) was then added to the reaction solution, and the mixture was extracted three times with CH_2Cl_2. The combined organic layers were washed with H_2O (200 ml), dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20:1) to give **23** (7.01 g, 29.8 mmol, 74%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CH_2CH_3), 2.15 (2H, m, pyrrolidine), 3.34 (1H, br d, pyrrolidine), 3.45 (1H, dt, pyrrolidine), 3.56 (2H, m, pyrrolidine), 4.32 (2H, q, CH_2CH_3), 4.65 (1H, m, pyrrolidine), 6.51 (2H, d, C_6H_4), 7.91 (2H, d, C_6H_4); TSPMS *m*/*z* 236 $(M+H)^+$.

5.30. Ethyl 4-{(3*S*)-aminopyrroridin-1-yl}benzoate (24a)

A mixture of **23** (200 mg, 0.850 mmol), phthalimide (250 mg, 1.70 mmol), and *n*-Bu_3P (0.424 ml, 1.70 mmol) in benzene (8.5 ml) was added 1,1′-(azodicarbonyl) dipiperidine (ADDP) (430 mg, 1.70 mmol) at 0 °C. After 15 h, the mixture was evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$/acetone = 50:1) to give ethyl 4-{(3*S*)-phthalimidepyrroridin-1-yl}benzoate (133 mg, 0.364 mmol, 43%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CH_2CH_3), 2.35 (1H, ddt, pyrrolidine), 2.81 (1H, dq, pyrrolidine), 3.50 (1H, q, pyrrolidine), 3.70 (2H, m, pyrrolidine), 3.83 (1H, t, pyrrolidine), 4.32 (2H, q, CH_2CH_3), 5.06 (1H, quintet, pyrrolidine), 6.53 (2H, d, C_6H_4), 7.75 (2H, m, phthalimide), 7.86 (2H, m, phthalimide), 7.93 (2H, d, C_6H_4); ESIMS *m*/*z* 365 $(M+H)^+$.

The above compound (1.50 g, 4.12 mmol) was suspended in MeOH (80 ml) and added hydrazine monohydrate 0.5 M (8.2 ml, 4.1 mmol). The mixture was stirred for 15 h at room temperature and then evaporated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/conc. NH_4OH =

30:10:1) to give **24a** (800 mg, 3.41 mmol, 83%) as a colorless solid.

5.31. 4-{(3*S*)-(Pyrimidin-2-ylamino)pyrroridin-1-yl}benzoic acid (26a)

Compound **24a** (1.02 g, 4.35 mmol) afforded **26a** (184 mg, 0.647 mmol, 58% in two steps) as a colorless solid according to **8a** from **3a**; ^{1}H NMR (400 MHz, CD_3OD) δ 2.04 (1H, m, pyrrolidine), 2.26 (1H, m, pyrrolidine), 3.23 (1H, dd, pyrrolidine), 3.35 (1H, m, pyrrolidine), 3.51 (1H, m, pyrrolidine), 3.63 (1H, dd, pyrrolidine), 4.51 (1H, dq, pyrrolidine), 6.52 (2H, br d, C_6H_4), 6.60 (1H, t, pyrimidine), 7.72 (2H, br d, C_6H_4), 8.30 (2H, d, pyrimidine); TSPMS *m*/*z* 285 $(M+H)^+$.

5.32. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-(pyrimidin-2-ylamino)pyrrolidin-1-yl}benzoylamino]propionic acid (25)

The title compound **25** (76.2 mg, 0.141 mmol, 79% in two steps) was synthesized from **26a** (100 mg, 0.352 mmol) as a colorless solid following the general procedure for **10** (step 1, 2); ^{1}H NMR (400 MHz, CD_3OD) δ 2.12 (1H, m, pyrrolidine), 2.40 (1H, dq, pyrrolidine), 3.30 (1H, m, pyrrolidine), 3.51 (3H, m, pyrrolidine and CONHCH_2CH), 3.67 (1H, dd, CONHCH_2CH), 3.75 (1H, dd, pyrrolidine), 4.14 (1H, dd, CONHCH$_2$C*H*), 4.63 (1H, dq, pyrrolidine), 6.57 (2H, d, C_6H_4), 6.65 (1H, t, pyrimidine), 7.41 (2H, br t, C_6H_5), 7.48 (1H, br t, C_6H_5), 7.62 (2H, d, C_6H_4), 7.81 (2H, m, C_6H_5), 8.30 (2H, d, pyrimidine); TSPMS *m*/*z* 511 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{24}H_{26}N_6O_5S$: 511.1764, found: 511.1770; $[\alpha]_D^{23}$ 22° (*c* 0.94, MeOH).

5.33. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)pyrrolidin-1-yl}benzoylamino]propionic acid (21)

The title compound **21** (29.3 mg, 0.0470 mmol, 42%) was synthesized from **25** (57.7 mg, 0.105 mmol) as a colorless solid following the general procedure for **10** (step 3); ^{1}H NMR (400 MHz, CD_3OD) δ 1.97 (2H, quintet, tetrahydropyrimidine), 2.08 (1H, m, pyrrolidine), 2.36 (1H, dt, pyrrolidine), 3.29 (1H, m, pyrrolidine), 3.38 (4H, t, tetrahydropyrimidine), 3.43 (1H, m, pyrrolidine), 3.51 (2H, m, pyrrolidine and CONHCH$_2$CH), 3.64 (2H, m, pyrrolidine and CONHCH$_2$CH), 4.19 (1H, dd, CONHCH$_2$CH), 4.23 (1H, q, pyrrolidine), 6.59 (2H, d, C_6H_4), 7.48 (3H, m, C_6H_5), 7.65 (2H, m, C_6H_4), 7.81 (2H, m, C_6H_5); FAB-MS *m*/*z* 515 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{24}H_{30}N_6O_5S$: 515.2077, found: 515.2076.

5.34. 4-{(3*S*)-(Pyridin-2-ylamino)piperidin-1-yl}benzoic acid (29a)

Under an argon atmosphere, toluene (0.62 ml) was added to a mixture of **3a** (20.0 mg, 0.0805 mmmol), 2-chloropyridine (7.0 μl, 0.0725 mmol), palladium (II) acetate (1.8 mg, 0.00805 mmol), (*R*)-(+)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) (5.0 mg, 0.00805 mmol), and sodium *t*-butoxide (9.3 mg, 0.0966 mmol). Argon was blown into the mixture with ultrasonication for 5 min, and the reaction mixture was stirred at 70 °C for 5.5 h. Saturated aqueous NH_4Cl (20 ml) was then added to the reaction mixture, and the mixture was extracted three times with AcOEt. The combined organic layers were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The residue was purified by preparative thin-layer silica gel column chromatography (hexane/AcOEt = 1:2) to give ethyl 4-{(3*S*)-(pyridin-2-ylamino)piperidin-1-yl}benzoate (11.2 mg, 43%) as a colorless syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (3H, t, CH_2CH_3), 1.70 (2H, m, piperidine), 1.88 (1H, m, piperidine), 2.01 (1H, dddd, piperidine), 3.05 (1H, dd, piperidine), 3.19 (1H, ddd, piperidine), 3.49 (1H, m, piperidine), 3.81 (1H, dd, piperidine), 3.99 (1H, ddddd, piperidine), 4.32 (2H, q, CH_2CH_3), 6.40 (1H, d, pyridine), 6.58 (1H, m, pyridine), 6.90 (2H, br d, C_6H_4), 7.41 (1H, ddd, pyridine), 7.91 (2H, br d, C_6H_4), 8.11 (1H, dd, pyridine); EIMS *m*/*z* 325 (M^+).

THF (0.9 ml) and MeOH (0.3 ml) were added to the above ester (20.0 mg, 0.0615 mmol), and 1.0 M aqueous NaOH (0.3 ml) was added to the solution. The mixture was stirred at 40 °C for 3 h and then concentrated under reduced pressure. Water (2 ml) was added to the residue, and the solution was adjusted to pH 4 by the addition of 1.0 M aqueous HCl. The precipitated solid was collected by a glass filter, washed twice with water, and then dried to give **29a** (17.3 mg, 95%) as a colorless solid; TSPMS *m*/*z* 298 $(M+H)^+$.

5.35. 4-{(3*S*)-(6-Hydroxypyridin-2-ylamino)piperidin-1-yl}benzoic acid (30a)

Under an argon atmosphere, toluene (13 ml) was added to a mixture of **7a** (516 mg, 2.56 mmmol), 6-methoxy-2-chloropyridine (549 mg, 3.82 mmol), palladium (II) acetate (58.3 mg, 0.260 mmol), (*R*)-(+)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) (161 mg, 0.259 mmol), and sodium *t*-butoxide (296 mg, 3.08 mmol). Argon was blown into the suspension with ultrasonication for 5 min. The reaction mixture was stirred at 75 °C for 18 h. Saturated aqueous NH_4Cl was then added to the reaction mixture, and the mixture was extracted three times with AcOEt. The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to give 4-{(3*S*)-(6-methoxypyridin-2-ylamino)piperidin-1-yl}benzonitrile (418 mg, 53%) as a light yellow syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (1H, m, piperidine), 1.73 (1H, m, piperidine), 1.87 (1H, m, piperidine), 2.04 (1H, m, piperidine), 2.96 (1H, dd, piperidine), 3.12 (1H, ddd, piperidine), 3.56 (1H, ddd, piperidine), 3.85 (3H, s, OCH_3), 3.93 (2H, m, piperidine), 4.44 (1H, brd, NH), 5.96 (1H, dd, pyridine), 6.04 (1H, dd, pyridine), 6.88 (2H, d, C_6H_4), 7.33 (1H, m, pyridine), 7.45 (2H, m, C_6H_4); EIMS *m*/*z* 308 (M^+).

To the above nitrile (203 mg, 0.657 mmmol) was added 5.0 M aqueous HCl (10 ml) to prepare a suspension. The suspension was stirred under reflux for 18 h, and

the reaction solution was concentrated under reduced pressure to give **30a** as a crude compound (256 mg); ^{1}H NMR (400 MHz, CD_3OD) δ 1.55 (2H, m, piperidine), 1.77 (1H, m, piperidine), 1.99 (1H, m, piperidine), 2.72 (1H, m, piperidine), 2.92 (1H, m, piperidine), 3.82 (2H, m, piperidine), 4.06 (1H, m, piperidine), 5.88 (1H, d, pyridine), 6.05 (1H, d, pyridine), 6.96 (2H, d, C_6H_4), 7.29 (1H, dd, pyridine), 7.73(2H, d, C_6H_4); TSPMS *m*/*z* 314 $(M+H)^+$.

5.36. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-(pyridin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (27)

Compound **29a** (17.3 mg, 0.0582 mmol) afforded the amide (32.0 mg, 95%) as a colorless amorphous following the general procedure for **10** (step 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (9H, s, *t*-Bu), 1.65 (1H, m, piperidine), 1.74 (1H, m, piperidine), 1.88 (1H, m, piperidine), 1.99 (1H, m, piperidine), 3.01 (1H, dd, piperidine), 3.15 (1H, ddd, piperidine), 3.45 (1H, br ddd, piperidine), 3.61 (1H, ddd, CONHC*H*$_2$CH), 3.76 (1H, dd, piperidine), 3.85 (1H, m, CONHC*H*$_2$CH), 3.93 (1H, m, CONHCH$_2$C*H*), 3.97 (1H, ddddd, piperidine), 6.41 (1H, d, pyridine), 6.59 (1H, ddd, pyridine), 6.91 (2H, br d, C_6H_4), 7.42 (1H, ddd, pyridine), 7.48 (2H, m, C_6H_5), 7.56 (1H, tt, C_6H_5), 7.67 (2H, br d, C_6H_4), 7.86 (2H, m, C_6H_5), 8.11 (1H, m, pyridine); TSPMS *m*/*z* 580 $(M+H)^+$; $[\alpha]_D^{24}$ 66° (*c* 1.0, CH_2Cl_2).

CH_2Cl_2 (0.5 ml) was added to the above ester (32.0 mg, 0.0552 mmol). Trifluoroacetic acid (0.2 ml) was added to the solution, and the mixture was stirred at room temperature for 6.5 h. The reaction solution was concentrated under reduced pressure, and the residue was purified by preparative thin-layer silica gel column chromatography ($CHCl_3$/MeOH/conc. NH_4OH = 30:10:1) to give **27** (28.9 mg, 100%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.58 (1H, dddd, piperidine), 1.75 (1H, ddddd, piperidine), 1.90 (1H, m, piperidine), 2.05 (1H, dddd, piperidine), 2.91 (1H, dd, piperidine), 3.03 (1H, ddd, piperidine), 3.53 (1H, dd, CONHC*H*$_2$CH), 3.61 (1H, m, piperidine), 3.65 (1H, dd, CONHC*H*$_2$CH), 3.84 (2H, m, CONHCH$_2$C*H* and piperidine), 3.94 (1H, dddd, piperidine), 6.62 (1H, m, pyridine), 6.67 (1H, d, pyridine), 6.96 (2H, br d, C_6H_4), 7.43 (2H, br t, C_6H_5), 7.52 (2H, m, pyridine and C_6H_5), 7.65 (2H, br d, C_6H_4), 7.84 (2H, m, C_6H_5), 7.92 (1H, m, pyridine); FABMS *m*/*z* 524 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{26}H_{29}N_5O_5S$: 524.1968, found: 524.1978; $[\alpha]_D^{25}$ 39° (*c* 0.36, MeOH/conc. NH_4OH = 10:1).

5.37. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-(6-hydroxypyridin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (28)

Compound **30a** (256 mg) afforded **28** as a colorless syrup following the general procedure for **27**; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73 (2H, m, piperidine), 1.93 (2H, m, piperidine), 3.20 (1H, dd, piperidine), 3.31 (2H, m, piperidine), 3.56 (2H, m), 3.66 (2H, m), 3.81 (1H, dd), 5.62(1H, d, pyridine), 5.70(1H, dd, pyridine), 6.96 (2H, m, C_6H_4), 7.32 (1H, m, pyridine), 7.44 (2H, m, C_6H_5), 7.51 (1H, m, C_6H_5), 7.68 (2H, d, C_6H_4), 7.85 (2H, m, C_6H_5); TSPMS *m*/*z* 540 $(M+H)^+$.

5.38. 4-{(3*S*)-(Benzylureido)piperidin-1-yl}benzoic acid (32a)

Under an argon atmosphere, a solution of **3a** (53.0 mg, 0.213 mmmol) in CH_3CN (0.5 ml) was added benzyl isocyanate (549 mg, 3.82 mmol). The reaction mixture was stirred at room temperature for 23 h. Saturated aqueous NH_4Cl was then added to the reaction mixture, and the mixture was extracted three times with CH_2Cl_2. The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give ethyl 4-{(3*S*)-(benzylureido)piperidin-1-yl}benzoate (99.6 mg) as a colorless syrup.

THF (3.0 ml) and MeOH (1.0 ml) were added to the above ester (99.0 mg), and 1.0 M aqueous NaOH (1.0 ml) was added to the solution. The mixture was stirred at 40 °C for 3 h and was then concentrated under reduced pressure. Water (5 ml) and MeOH (5 ml) added to the residue, and the solution adjusted to pH 6 by the addition of 1.0 M aqueous HCl. The precipitated solid was collected by a glass filter, washed twice with water, and then dried to give **32a** (63.4 mg, 84% in two steps) as a colorless solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (1H, m, piperidine), 1.55 (1H, m, piperidine), 1.71 (1H, m, piperidine), 1.82 (1H, m, piperidine), 2.85 (1H, dd, piperidine), 3.03 (1H, m, piperidine), 3.61 (3H, m, piperidine), 4.20 (2H, d, $C_6H_5CH_2$), 6.91 (2H, d, C_6H_4), 7.27 (5H, m, $C_6H_5CH_2$), 7.73 (2H, d, C_6H_4); TSPMS *m*/*z* 354 $(M+H)^+$.

5.39. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-(benzylureido)piperidin-1-yl}benzoylamino]propionic acid (31)

Compound **32a** (30.0 mg, 0.0849 mmol) afforded **31** (46.3 mg, 92% in two steps) as a colorless amorphous following the general procedure for **27**; ^{1}H NMR (400 MHz, CD_3OD) δ 1.55 (1H, m, piperidine), 1.69 (1H, m, piperidine), 1.86 (2H, m, piperidine), 2.99 (1H, dd, piperidine), 3.13 (1H, m, piperidine), 3.44 (1H, m, piperidine), 3.55 (1H, dd, CONHCH$_2$CH), 3.64 (2H, m, piperidine and CONHCH$_2$CH), 3.75 (1H, dd, CONHCH$_2$CH), 3.83 (1H, m, piperidine), 4.32 (2H, s, $C_6H_5CH_2$), 6.96 (2H, d, C_6H_4), 7.26 (5H, m, $C_6H_5CH_2$), 7.45 (2H, t, C_6H_5), 7.52 (1H, m, C_6H_5), 7.68 (2H, d, C_6H_4), 7.85 (2H, m, C_6H_5); TSPMS *m*/*z* 580 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{29}H_{33}N_5O_6S$: 580.2230, found: 580.2238; $[\alpha]_D^{25}$ 69° (*c* 1.1, MeOH).

5.40. 4-[(3*S*)-{(*t*-Butoxycarbonyl)amino}piperidin-1-yl]benzoic acid (34a)

Compound **3a** (100 mg, 0.403 mmol) was added to DMF (4.0 ml). Et_3N (112 μl, 0.806 mmol) and di-*t*-butyl dicarbonate (111 μl, 0.483 mmol) were added to the solution, and the mixture was stirred at room temperature for 1.5 h. Saturated brine (50 ml) was added to the reaction solution, and the mixture was extracted three times with AcOEt. The combined organic layers

were washed twice with a mixed solution composed of saturated brine (50 ml) and water (50 ml), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude compound. Subsequently, THF (6.0 ml) and MeOH (2.0 ml) were added to the crude compound, and 1.0 M aqueous NaOH (2.0 ml) was added to the solution. The mixture was stirred at 40 °C for 18 h and then at 60 °C for 10 h, and then concentrated under reduced pressure. Water (3 ml) was added to the residue. The solution was then adjusted to pH 7 by the addition of 1.0 M aqueous HCl. The precipitated solid was collected by a glass filter, washed twice with water, and then dried to give **34a** (118.3 mg, 92%) as a colorless solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.40 (9H, s, *t*-Bu), 1.45 (2H, m, piperidine), 1.72 (1H, m, piperidine), 1.83 (1H, m, piperidine), 2.50 (1H, m, piperidine), 2.72 (1H, br dd, piperidine), 2.84 (1H, ddd, piperidine), 3.39 (1H, m, piperidine), 3.75 (1H, m, piperidine), 6.91 (2H, d, C_6H_4), 7.74 (2H, d, C_6H_4); EIMS *m*/*z* 320 $(M)^+$.

5.41. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-guanidinopiperidin-1-yl}benzoylamino]propionic acid (33)

Compound **34a** (80.0 mg, 0.250 mmol) afforded the amide (150 mg, 100%) as a colorless syrup following the general procedure for **10** (step 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (9H, s, *t*-Bu), 1.47 (9H, s, Boc), 1.55 (1H, m, piperidine), 1.69 (1H, m, piperidine), 1.80 (1H, m, piperidine), 1.86 (1H, m, piperidine), 3.06 (1H, m, piperidine), 3.18 (1H, m, piperidine), 3.31 (1H, m, piperidine), 3.59 (2H, m, piperidine or CONHCH_2CH), 3.81 (1H, m, piperidine), 3.90 (2H, m, piperidine or CONHCH_2C*H*), 6.91 (2H, d, C_6H_4), 7.49 (2H, br t, C_6H_5), 7.57 (1H, br t, C_6H_5), 7.68 (2H, d, C_6H_4), 7.86 (2H, m, C_6H_5); FABMS *m*/*z* 602 $(M+H)^-$.

CH_2Cl_2 (2.0 ml) was added to the above compound (109.0 mg, 0.181 mmol), and the solution was then ice cooled, to which trifluoroacetic acid (0.4 ml) was added. The mixture was stirred at that temperature for 5.5 h. The reaction solution was poured into a mixed solvent composed of MeOH (10 ml) and conc. NH_4OH (10 ml) which had been cooled to 0 °C. The mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/conc. NH_4OH = 30:10:1) to give *t*-butyl 3-[4-{(3*S*)-aminopiperidin-1-yl}benzoylamino]-(2*S*)-(benzenesulfonylamino)propionate (90 mg, 100%) as a colorless amorphous; ^{1}H NMR (400 MHz, CD_3OD) δ 1.23 (9H, s, *t*-Bu), 1.42 (1H, dddd, piperidine), 1.68 (1H, m, piperidine), 1.85 (1H, m, piperidine), 2.00 (1H, m, piperidine), 2.78 (1H, dd, piperidine), 2.96 (2H, m, piperidine), 3.49 (1H, dd, CONHCH_2CH), 3.61 (1H, m, piperidine), 3.65 (1H, dd, CONHCH_2CH), 3.75 (1H, br dd, piperidine), 4.11 (1H, dd, CONHCH$_2$C*H*), 6.96 (2H, d, C_6H_4), 7.47 (2H, br t, C_6H_5), 7.54 (1H, br t, C_6H_5), 7.67 (2H, d, C_6H_4), 7.83 (2H, m, C_6H_5); TSPMS *m*/*z* 503 $(M+H)^+$.

1,4-Dioxane (0.36 ml) and water (0.36 ml) were added to the above amine (82.2 mg, 0.164 mmol). 1*H*-Pyrazole-1-carboxamidine hydrochloride (105 mg, 0.719 mmol) and *i*-Pr_2EtN (0.120 ml, 0.689 mmol) were added to the solution, and the mixture was vigorously stirred at room temperature for 18 h. The reaction solution was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/conc. NH_4OH = 9:3:0.3) to give *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{(3*S*)-guanidinopiperidin-1-yl}benzoylamino]propionate (50.1 mg, 56%) as a light yellow solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.23 (9H, s, *t*-Bu), 1.67 (1H, m, piperidine), 1.76 (1H, m, piperidine), 1.93 (2H, m, piperidine), 3.20 (2H, m, piperidine), 3.37 (1H, m, piperidine), 3.49 (1H, dd, CONHCH_2CH), 3.57 (1H, m, piperidine), 3.65 (1H, dd, CONHCH_2CH), 3.77 (1H, m, piperidine), 4.12 (1H, dd, CONHCH$_2$C*H*), 7.00 (2H, m, C_6H_4), 7.48 (2H, m, C_6H_5), 7.55 (1H, m, C_6H_5), 7.69 (2H, m, C_6H_4), 7.84 (2H, m, C_6H_5); FABMS *m*/*z* 545 $(M+H)^+$; $[\alpha]_D^{26}$ 22° (*c* 1.5, MeOH).

CH_2Cl_2 (0.5 ml) was added to above compound (18.4 mg, 0.0338 mmol), to which trifluoroacetic acid (0.5 ml) was added. The mixture was stirred at room temperature for 18 h. The reaction solution was concentrated under reduced pressure to give a trifluoroacetate of the title compound. The residue was purified by preparative thin-layer silica gel column chromatography (CH_2Cl_2/EtOH/conc. NH_4OH/water = 8:8:1:1) and then purified by Sephadex LH-20 (MeOH) to give **33** (15.5 mg, 94%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.71 (2H, m, piperidine), 1.92 (2H, m, piperidine), 3.15 (1H, m, piperidine), 3.21 (1H, m, piperidine), 3.38 (1H, m, piperidine), 3.47 (2H, m, piperidine and CONHCH_2CH), 3.62 (1H, m, CONHCH_2CH), 3.75 (1H, m, piperidine), 3.92 (1H, m, CONHCH$_2$C*H*), 6.99 (2H, d, C_6H_4), 7.44 (2H, m, C_6H_5), 7.51 (1H, m, C_6H_5), 7.69 (2H, d, C_6H_4), 7.83 (2H, d, C_6H_5); TSPMS *m*/*z* 489 $(M+H)^+$; $[\alpha]_D^{26}$ 66° (*c* 0.097, MeOH).

5.42. 4-{(3*R*)-(Pyrimidin-2-ylamino)pyrroridin-1-yl}benzoic acid (36)

Enantiomer of **23** (*ent*-**23**) was prepared as per the following method.

A mixture of **23** (3.00 g, 12.7 mmol), AcOH (920 mg, 15.3 mmol), and Ph_3P (4.00 g, 15.3 mmol) in THF (60 ml) was added diethyl azodicarboxylate (DEAD) (2.36 ml, 15.3 mmol) at 0 °C. After stirring for 15 h at room temperature, H_2O (200 ml) was then added to the reaction mixture, and the mixture was extracted three times with AcOEt (200 ml). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 3:1) to give ethyl 4-{(3*S*)-acetoxypiperidin-1-yl}benzoate (2.75 g, 9.91 mmol, 78%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CH_2CH_3), 2.06 (3H, S, CH_3CO_2), 2.26 (2H, m, pyrrolidine), 3.42 (1H, d, pyrrolidine), 3.50 (2H, m, pyrrolidine), 3.67 (1H, dd, pyrrolidine), 4.32 (2H, q, CH_2CH_3), 5.44 (1H, m, pyrrolidine), 6.52 (2H, br d, C_6H_4), 7.93 (2H, br d, C_6H_4).

To the above acetate (2.70 g, 9.73 mmol) in EtOH (50 ml), NaOEt (795 mg, 11.7 mmol) was added. The solution was stirred for 3.5 h. The reaction mixture was poured into a mixture of saturated aqueous NH_4Cl (800 ml) and CH_2Cl_2 (800 ml) with stirring. The mixture was extracted three times with CH_2Cl_2 (500 ml). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/acetone = 20:1) to give *ent*-**23** (2.24 g, 9.54 mmol, 98%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CH_2CH_3), 2.15 (2H, m, pyrrolidine), 3.34 (1H, br d, pyrrolidine), 3.45 (1H, dt, pyrrolidine), 3.56 (2H, m, pyrrolidine), 4.32 (2H, q, CH_2CH_3), 4.65 (1H, m, pyrrolidine), 6.51 (2H, d, C_6H_4), 7.91 (2H, d, C_6H_4); APCIMS *m*/*z* 236 $(M+H)^+$.

Compound **36** (156 mg, 0.549 mmol, 33% in four steps) was prepared from *ent*-**23** (1.84 g, 7.82 mmol) using the procedures described for preparing **26a** via **24a** from **23**; ^{1}H NMR (400 MHz, CD_3OD) δ 2.04 (1H, m, pyrrolidine), 2.26 (1H, m, pyrrolidine), 3.23 (1H, dd, pyrrolidine), 3.35 (1H, m, pyrrolidine), 3.51 (1H, m, pyrrolidine), 3.63 (1H, dd, pyrrolidine), 4.51 (1H, dq, pyrrolidine), 6.52 (2H, br d, C_6H_4), 6.60 (1H, t, pyrimidine), 7.72 (2H, br d, C_6H_4), 8.30 (2H, d, pyrimidine); TSPMS *m*/*z* 285 $(M+H)^+$.

5.43. (2*S*)-Benzenesulfonylamino-3-[4-{(3*R*)-(pyrimidin-2-ylamino)pyrrolidin-1-yl}benzoylamino]propionic acid (35)

The title compound **35** (99.0 mg, 0.194 mmol, 100% in two steps) was synthesized from **36** (100 mg, 0.352 mmol) as a colorless solid following the general procedure for **10** (step 1, 2); ^{1}H NMR (400 MHz, CD_3OD) δ 2.16 (1H, ddt, pyrrolidine), 2.41 (1H, dddd, pyrrolidine), 3.31 (1H, m, pyrrolidine), 3.44 (1H, m, pyrrolidine), 3.50 (1H, dd, $CONHCH_2CH$), 3.55 (1H, m, pyrrolidine), 3.69 (1H, dd, $CONHCH_2CH$), 3.72 (1H, dd, pyrrolidine), 4.16 (1H, dd, $CONHCH_2CH$), 4.68 (1H, tt, pyrrolidine), 6.55 (2H, d, C_6H_4), 6.83 (1H, t, pyrimidine), 7.40 (2H, m, C_6H_5), 7.48 (1H, tt, C_6H_5), 7.62 (2H, br d, C_6H_4), 7.81 (2H, m, C_6H_5), 8.46 (2H, br d, pyrimidine); FABMS *m*/*z* 511 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{24}H_{26}N_6O_5S$: 511.1764, found: 511.1768; $[\alpha]_D^{23}$ 98° (*c* 0.17, MeOH/CH_2Cl_2 = 1:1).

5.44. 3-Fluoro-4-{(3*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (bf 38)

Compound **38** (280 mg, 0.886 mmol) was prepared from D-ornithine as a colorless solid using the procedures described for preparing **8b** via **3b** from L-ornithine; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.50 (1H, m, piperidine), 1.66 (1H, m, piperidine), 1.82 (1H, m, piperidine), 1.94 (1H, m, piperidine), 2.63 (1H, m, piperidine), 2.73 (1H, br t, piperidine), 3.30 (1H, m, piperidine), 3.56 (1H, br d, piperidine), 3.99 (1H, m, piperidine), 6.57 (1H, t, pyrimidine), 7.05 (1H, t, C_6H_3), 7.49 (1H, dd, C_6H_3), 7.61 (1H, dd, C_6H_3), 8.28 (2H, d, pyrimidine), EIMS *m*/*z* 316 (M^+).

5.45. (2*S*)-Benzenesulfonylamino-3-[3-fluoro-4-{(3*R*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (37)

The title compound **37** (46.0 mg, 62% in three steps) was synthesized from **38** (140 mg, 0.44 mmol) as a colorless solid following the general procedure for **10**; ^{1}H NMR (400 MHz, CD_3OD) δ 1.67 (1H, m, piperidine), 1.77 (1H, m, piperidine), 1.90 (2H, m, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 3.11 (3H, m, piperidine), 3.28 (1H, m, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.52 (1H, dd, $CONHCH_2CH$), 3.68 (1H, dd, $CONHCH_2CH$), 3.72 (1H, m, piperidine), 3.89 (1H, dd, $CONHCH_2CH$), 7.04 (1H, dd, C_6H_3), 7.50 (5H, m, C_6H_3 and C_6H_5), 7.85 (2H, m, C_6H_5); TSPMS *m*/*z* 547 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}N_6O_5FS$: 547.2139, found: 547.2148.

5.46. 4-[2-{(Pyrimidin-2-ylamino)methyl}pyrrolidin-1-yl]benzoic acid (40)

Compound **40** (120 mg 38% in two steps) was prepared from racemic **2** as a colorless solid using the procedures described for preparing **8a** from **2** via **3a**; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.85 (1H, m, pyrrolidine), 2.02 (3H, m, pyrrolidine), 2.94 (1H, ddd, $NHCH_2$), 3.13 (1H, m, $NHCH_2$), 3.46 (1H, br t, pyrrolidine), 3.59 (1H, m, pyrrolidine), 3.94 (1H, m, pyrrolidine), 6.60 (1H, t, pyrimidine), 6.95 (2H, d, C_6H_4), 7.77 (2H, d, C_6H_4), 8.35 (2H, m, pyrimidine); EIMS *m*/*z* 298 $(M)^+$.

5.47. (2*S*)-Benzenesulfonylamino-3-[4-[2-{(1,4,5,6-tetrahydropyrimidin-2-ylamino)methyl}pyrrolidin-1-yl]benzoylamino]propionic acid (39)

The title compound **39** (67.2 mg, 77% in three steps) was synthesized from **40** (88.0 mg, 0.295 mmol) as a colorless solid following the general procedure for **10**; ^{1}H NMR (400 MHz, CD_3OD) δ 1.83 (2H, quintet, tetrahydropyrimidine), 1.89 (1H, m, pyrrolidine), 2.10 (3H, m, pyrrolidine), 3.29 (7H, m, pyrrolidine, tetrahydropyrimidine and $NHCH_2$), 3.56 (2H, m, pyrrolidine and $CONHCH_2CH$), 3.68 (2H, m, pyrrolidine and $CONHCH_2CH$), 4.10 (1H, m, $CONHCH_2CH$), 6.69 (2H, d, C_6H_4), 7.50 (2H, t, C_6H_5), 7.56 (1H, t, C_6H_5), 7.72 (2H, d, C_6H_4), 7.87 (2H, m, C_6H_5); TSPMS *m*/*z* 529 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_5S$: 529.2233, found: 529.2226; $[\alpha]_D^{26}$ 83° (*c* 0.22, MeOH/conc. NH_4OH = 10:1).

5.48. *t*-Butyl (2*S*)-(benzyloxycarbonyl)amino-3-[3-fluoro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (41b)

Compound **8b** (75 mg, 0.24 mmol) and *t*-butyl (2*S*)-*N*-benzyloxycarbonyl-2,3-diaminopropionate hydrochloride **9b** (85 mg, 0.29 mmol) afforded **41b** (140 mg, 100%) as a colorless solid following the general procedure for **10** (step 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (9H, s, *t*-Bu), 1.75 (2H, m, piperidine), 1.92 (2H, m, piperidine), 3.10 (1H, m, piperi-

dine), 3.14 (1H, m, piperidine), 3.42 (1H, m, piperidine), 3.79 (3H, m, CONHCH_2CH and piperidine), 4.25 (1H, m, piperidine), 4.44 (1H, m, CONHCH$_2$*CH*), 5.10 (2H, s, $CH_2C_6H_5$), 5.58 (1H, d, NH), 5.83 (1H, d, NH), 6.51 (1H, t, pyrimidine), 6.84 (1H, brs, NH), 6.97 (1H, dd, C_6H_3), 7.32 (7H, m, C_6H_3 and C_6H_5), 8.27 (2H, d, pyrimidine); FAB-MS *m*/*z* 593 $(M+H)^+$.

5.49. *t*-Butyl (2*S*)-amino-3-[3-fluoro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (42b)

THF was added to **41b** (128 mg, 0.21 mmol). To the solution 5% palladium on charcoal (100 mg) was added. The mixture was vigorously stirred under a hydrogen pressure of 1 atm at room temperature for 12 h. The reaction mixture was filtered through Celite, followed by washing with THF and EtOH. The filtrate and the washings were combined, and the combined solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 8:2) to give **42b** (80.0 mg, 83%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (9H, s, *t*-Bu), 1.76 (2H, m, piperidine), 1.89 (2H, m, piperidine), 3.14 (3H, m, piperidine), 3.43 (2H, m, piperidine and CONHCH_2CH), 3.57 (1H, m, CONHCH_2CH), 3.79 (1H, m, CONHCH$_2$*CH*), 4.24 (1H, m, piperidine), 5.55 (1H, d, NH), 6.52 (1H, t, pyrimidine), 6.68 (1H, m, NH), 6.97 (1H, dd, C_6H_3), 7.45 (2H, m, C_6H_3), 8.27 (2H, d, pyrimidine); EIMS *m*/*z* 458 (M^+).

5.50. *t*-Butyl (2*S*)-amino-3-[4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (42a)

The title compound **42a** was synthesized from **8a** (60.0 mg, 0.20 mmol) following the general procedure for afforded **42b**; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (9H, s, *t*-Bu), 1.76 (2H, m, piperidine), 1.86 (1H, m, piperidine), 2.00 (1H, m, piperidine), 3.03 (1H, dd, piperidine), 3.17 (1H, ddd, piperidine), 3.45 (2H, m, piperidine), 3.60 (1H, m, CONHCH_2CH), 3.80 (2H, m, CONHCH_2*CH*), 4.15 (1H, m, piperidine), 5.29 (1H, d, NH), 6.54 (1H, t, pyrimidine), 6.67 (1H, brs, NH), 6.92 (2H, d, C_6H_4), 7.66 (2H, d, C_6H_4), 8.28 (2H, d, pyrimidine); TSPMS *m*/*z* 441 $(M+H)^+$.

5.51. *t*-Butyl 3-[3-fluoro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-methoxy-benzenesulfonyl)amino}propionate (43b; $R_3 = C_6H_4$-4-OMe)

DMF (3.0 ml) was added to **42b** (70 mg, 0.15 mmol). *i*-Pr_2EtN (0.055 ml, 0.30 mmol) and 4-methoxybenzenesulfonyl chloride (31 mg, 0.15 mmol) were added to the solution, and the mixture was stirred at room temperature for 3 h. Piperidine was added to the reaction solution, water and aqueous $NaHCO_3$ were then added, and the mixture was extracted three times with AcOEt. The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 8:2) to give **43b** ($R_3 = C_6H_4$-4-OMe) (80 mg, 83%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (9H, s, *t*-Bu), 1.77 (2H, m, piperidine), 1.92 (2H, m, piperidine), 3.09 (1H, dd, piperidine), 3.15 (2H, m, piperidine), 3.43 (1H, dd, piperidine), 3.52 (1H, ddd, CONHCH_2CH), 3.84 (3H, s, OMe), 3.90 (2H, m, CONHCH_2*CH*), 4.25 (1H, m, piperidine), 5.56 (1H, d, NH), 6.52 (1H, t, pyrimidine), 6.61 (1H, brs, NH), 6.94 (2H, d, C_6H_4), 6.98 (1H, dd, C_6H_3), 7.48 (2H, m, C_6H_3), 7.76 (2H, d, C_6H_4), 8.28 (2H, d, pyrimidine); TSPMS *m*/*z* 629 $(M+H)^+$.

5.52. 3-[3-Fluoro-4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-methoxybenzenesulfonyl)amino}propionic acid (44)

Compound **43b** ($R_3 = C_6H_4$-4-OMe) (30.0 mg, 0.048 mmol) afforded **44** (20.0 mg, 72% in two steps) as a colorless solid following the general procedure for **10** (step 2 and 3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67 (1H, m, piperidine), 1.78 (1H, m, piperidine), 1.91 (2H, m, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 3.12 (3H, m, piperidine), 3.27 (1H, m, piperidine), 3.36 (4H, t, tetrahydropyrimidine), 3.48 (1H, dd, CONHCH_2CH), 3.68 (1H, dd, CONHCH_2CH), 3.72 (1H, m, piperidine), 3.80 (3H, s, OMe), 3.85 (1H, dd, CONHCH$_2$*CH*), 6.94 (2H, d, C_6H_4), 7.05 (1H, dd, C_6H_3), 7.48 (1H, dd, C_6H_3), 7.53 (1H, dd, C_6H_3), 7.74 (2H, d, C_6H_4); TSPMS *m*/*z* 577 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{26}H_{33}N_6O_6FS$: 577.2245, found: 577.2251.

5.53. 3-[3-Fluoro-4-{(3*S*)-(1,4,5,6-tetrahydro-pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-hydroxybenzenesulfonyl)amino}propionic acid (49)

1,2-Dichloroethane (7.0 ml) was added to 3-[3-fluoro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-methoxy-benzenesulfonyl)amino}propionic acid (45.0 mg, 0.07 mmol) (obtained as a intermediate of **44** from **43b**). 1.0 M solution of boron tribromide in dichloromethane (0.700 ml, 0.700 mmol) was then added to the solution, and the mixture was stirred at 40 °C for 4 h. The reaction solution was concentrated under reduced pressure, and the residue was then purified by preparative silica gel column chromatography ($CHCl_3$/MeOH/conc. NH_4OH = 8:2:0.2) to give 3-[3-fluoro-4-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-hydroxy-benzenesulfonyl)amino}propionic acid (39 mg, 100%) as a colorless solid; FABMS *m*/*z* 559 $(M+H)^+$.

THF (3.5 ml), water (1.0 ml), and acetic acid (0.5 ml) were added to the above compound (45.0 mg, 0.08 mmol). To the solution 5% palladium on charcoal (50 mg) was added. The mixture was vigorously stirred under a hydrogen pressure of 1 atm at room temperature for 15 h. The reaction solution was filtered through Celite, followed by washing with THF and EtOH. The filtrate and the washings were combined, and the combined solution was concentrated under reduced pressure. The residue was purified by preparative thin-layer silica gel column chromatography ($CHCl_3$/MeOH/conc. NH_4OH = 8:2:0.2) and then purified by Sephadex LH-20 (MeOH) to give **49** as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.67 (1H, m, piperidine), 1.80 (1H, m, piperidine), 1.95 (4H, m, tetrahydro-

pyrimidine and piperidine), 3.15 (2H, m, piperidine), 3.38 (4H, t, tetrahydropyrimidine), 3.49 (1H, dd, CONHCH$_2$CH), 3.69 (1H, dd, CONHCH$_2$CH), 3.73 (1H, m, piperidine), 3.86 (1H, dd, CONHCH$_2$CH), 6.95 (2H, d, C$_6$H$_4$), 7.07 (1H, t, C$_6$H$_3$), 7.50 (1H, dd, C$_6$H$_3$), 7.55 (1H, dd, C$_6$H$_3$), 7.96 (2H, d, C$_6$H$_4$); TSPMS *m*/*z* 563 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}N_6O_6FS$: 563.2088, found 563.2085.

The following compounds were prepared using the procedures described for preparing **44** from **8b**.

5.54. 3-[4-{(3*S*)-(Pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(thiophene-2-sulfonyl)amino}propionic acid (45)

Amine **42a** (40.0 mg, 0.0900 mmol) and 2-thiophenesulfonyl chloride (16.0 mg, 0.0900 mmol) afforded **45** (24.0 mg, 49% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD$_3$OD) δ 1.58 (1H, m, piperidine), 1.70 (1H, m, piperidine), 1.86 (1H, m, piperidine), 1.94 (3H, m, tetrahydropyrimidine and piperidine), 3.02 (1H, dd, J = 7.6, 12.4 Hz, piperidine), 3.08 (1H, m, piperidine), 3.34 (4H, t, J = 5.8 Hz, tetrahydropyrimidine), 3.40 (1H, m, piperidine), 3.54 (2H, m, CONH*CH*$_2$CH and piperidine), 3.63 (1H, m, piperidine), 3.67 (1H, dd, J = 5.1, 13.4 Hz, CONH*CH*$_2$CH), 3.97 (1H, dd, J = 5.1, 8.3 Hz, CONHCH$_2$*CH*), 6.94 (2H, d, J = 9.0 Hz, C$_6$H$_4$), 7.02 (1H, dd, J = 3.9, 5.1 Hz, thiophene), 7.57 (1H, dd, J = 1.5, 3.9 Hz, thiophene), 7.66 (3H, m, thiophene and C$_6$H$_4$); ^{13}C NMR (DMSO-d_6) δ 19.7, 22.8, 30.3, 37.8, 42.5, 46.3, 47.3, 52.3, 55.8, 114.1, 123.7, 127.5, 128.1, 131.6, 132.3, 141.7, 152.2, 152.3, 165.5, 173.0; TSPMS *m*/*z* 535 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{23}H_{30}N_6O_5S_2$: 535.1797, found: 535.1797; $[\alpha]_D^{21.5}$ 86° (*c* 0.50, MeOH).

5.55. (2*S*)-(Pyridine-3-sulfonyl)amino-3-[4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (46)

Amine **42a** (105 mg, 0.238 mmol) and 3-pyridinesulfonyl chloride afforded **46** (8.6 mg mg, 8.5% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD$_3$OD) δ 1.61 (1H, dddd, piperidine), 1.67–1.77 (1H, m, piperidine), 1.81–2.00 (2H, m, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 3.06 (1H, dd, piperidine), 3.14 (1H, ddd, piperidine), 3.34–3.42 (5H, m, tetrahydropyrimidine and piperidine), 3.53 (1H, br d, piperidine), 3.57 (1H, dd, CONHCH$_2$), 3.64 (1H, dd, CONHCH$_2$), 3.60–3.71 (1H, m, piperidine), 3.85 (1H, dd, CONHCH$_2$CH), 6.95 (2H, d, C$_6$H$_4$), 7.50 (1H, ddd, pyridine), 7.68 (2H, d, C$_6$H$_4$), 8.24 (1H, ddd, pyridine), 8.65 (1H, dd, pyridine), 8.97 (1H, dd, pyridine); FABMS *m*/*z* 530 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{24}H_{31}N_7O_5S$: 530.2186, found: 530.2180.

5.56. (2*S*)-(Butane-1-sulfonyl)amino-3-[4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (47)

Amine **42a** (104 mg, 0.235 mmol) and 1-butanesulfonyl chloride (49 μl, 0.376 mmol) afforded **47** (4.3 mg, 3.9% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD$_3$OD) δ 0.92 (3H, t, *n*-Bu), 1.41 (2H, tq, *n*-Bu), 1.57–1.94 (6H, m, piperidine and *n*-Bu), 1.97 (2H, quintet, tetrahydropyrimidine), 3.06 (1H, ddd, piperidine), 3.04–3.15 (2H, m, *n*-Bu), 3.19 (1H, ddd, piperidine), 3.36 (5H, m, tetrahydropyrimidine and piperidine), 3.53 (1H, dd, piperidine), 3.57 (1H, dd, CONHCH$_2$), 3.62–3.68 (1H, m, piperidine), 3.72 (1H, dd, CONHCH$_2$), 3.95 (1H, dd, CONHCH$_2$CH), 6.98 (2H, d, C$_6$H$_4$), 7.73 (2H, d, C$_6$H$_4$); TSPMS *m*/*z* 509 $(M+H)^+$; $[\alpha]_D^{24}$ 8.5° (*c* 0.12, MeOH).

5.57. (2*S*)-(3-Acetoxypropane-1-sulfonyl)amino-3-[4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (48)

Amine **42a** (100 mg, 0.227 mmol) and 3-acetoxy-1-propanesulfonyl chloride (126 mg, 0.630 mmol) afforded **48** (17.7 mg, 26% in three steps) as a colorless solid; ^{1}H NMR (400 MHz, CD$_3$OD) δ 1.59 (1H, dddd, piperidine), 1.65–1.76 (1H, m, piperidine), 1.81–2.00 (2H, m, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 2.02 (3H, s, Ac), 2.02 (2H, m, Pr), 3.04 (1H, dd, piperidine), 3.07–3.20 (3H, m, piperidine and Pr), 3.33–3.40 (5H, m, tetrahydropyrimidine and piperidine), 3.52 (1H, dd, piperidine), 3.58 (1H, dd, CONHCH$_2$), 3.59–3.68 (1H, m, piperidine), 3.70 (1H, dd, CONHCH$_2$), 3.99 (1H, dd, CONHCH$_2$CH), 4.09 (2H, t, Pr), 6.94 (2H, d, C$_6$H$_4$), 7.71 (2H, d, C$_6$H$_4$); FABMS *m*/*z* 553 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{24}H_{36}N_6O_7S$: 553.2444, found: 553.2441.

5.58. (2*S*)-(3-Hydroxypropane-1-sulfonyl)amino-3-[4-{(3*S*)-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (50)

THF (0.8 ml) and MeOH (0.2 ml) were added to *t*-butyl (2*S*)-(3-acetoxypropane-1-sulfonyl)amino-3-[4-{(3*S*)-(pyrimidin-2-ylamino)-piperidin-1-yl}benzoylamino]propionate (obtained as the intermediate of **48** from **42a**) (50.0 mg, 0.0828 mmol). LiOH (3.97 mg, 0.166 mmol) was added to the solution, and the mixture was stirred at room temperature for 3 h. The reaction solution was adjusted to pH 5 by the addition of 0.1 M aqueous HCl and then concentrated under reduced pressure. The residue was purified by preparative thin-layer silica gel column chromatography (hexane/AcOEt = 1:10) to give *t*-butyl (2*S*)-(3-hydroxypropane-1-sulfonyl)amino-3-[4-{(3*S*)-(pyrimidin-2-ylamino)-piperidin-1-yl}benzoylamino]propionate (34.0 mg, 73%) as a colorless solid; FABMS *m*/*z* 563 $(M+H)^+$.

Compound **50** was prepared from the above propionate as a colorless solid using the procedures described for preparing **10** (step 2, 3); ^{1}H NMR (400 MHz, CD$_3$OD) δ 1.62 (1H, m, piperidine), 1.73 (1H, m, piperidine), 1.95 (6H, m, piperidine, tetrahydropyrimidine and Pr), 3.10 (1H, dd, piperidine), 3.15 (2H, t, Pr), 3.18 (1H, m, piperidine), 3.35 (1H, m, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.54 (1H, br dd, piperidine), 3.57 (1H, dd, CONHC*H*$_2$CH), 3.60 (2H, t, Pr), 3.65 (1H, m, piperidine), 3.73 (1H, dd, CONHC*H*$_2$CH), 3.98 (1H, dd, CONHCH$_2$C*H*), 6.98 (2H, d, C$_6$H$_4$), 7.73 (2H, d, C$_6$H$_4$); FAB-

MS *m/z* 511 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{22}H_{34}N_6O_6S$: 511.2339, found 511.2348.

5.59. Integrin-binding assays

Compounds were evaluated for their inhibitory activities in $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$-ELISA (enzyme-linked immunosorbent assay). $\alpha_v\beta_3$[22] was purified from human placenta, using RGDSPK-Sepharose CL-4B chromatography, followed by mono Q chromatography (Pharmacia). $\alpha_{IIb}\beta_3$[22] was purified from human platelet by RGDSPK-Sepharose CL-4B. $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ binding assays were performed according to the modified method of Kouns et al.[23] EIA plates (Nunc) were coated with $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ and blocked with bovine serum albumin. In each reaction, the reaction mixture (20 mM Tris–HCl, 150 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4, 100 μl) including vitronectin (Calbiochem) or fibrinogen, added to the receptor-coated plate, was incubated for 4 h at 25 °C. Thereafter the ligand binding was measured using anti-vitronectin rabbit antibody (Calbiochem) and peroxidase-conjugated anti-rabbit IgG antibody (Capell) for $\alpha_v\beta_3$, or peroxidase-conjugated anti-fibrinogen antibody (Capell) for $\alpha_{IIb}\beta_3$, and 2, 2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) as the substrate of peroxidase. The IC_{50} values were determined from measurement of absorbance at 415 nm.

5.60. Adhesion of human aorta smooth muscle cells to vitronectin

The adhesion of human aorta smooth muscle cells to vitronectin was measured as described before.[24] Briefly EIA plates (Nunc) were coated with human vitronectin (Calbiochem) and blocked with bovine serum albumin. The cell suspension of human aorta smooth muscle cells (50,000 cells/100 μl, Clonetics) in Dulbecco's modified Eagle's basal medium containing 0.1% bovine serum albumin was added to the vitronectin-coated plates and incubated for 1.5 h at 37 °C in the presence or absence of the test compounds. The adherent cells were stained with toluidine blue and calculated by measuring of absorbance at 405 nm after the cytolysis by SDS. The IC_{50} values were determined graphically from two or more independent experiments.

5.61. Platelet aggregation assay

Platelet aggregation was determined according to the previous method.[23] Human platelet-rich plasma obtained from healthy volunteers was prepared and the aggregation was induced with 5μM ADP. The IC_{50} values were determined from two independent experiments.

5.62. Aqueous solubility

Aqueous solubility of compounds was determined in water at room temperature. An excess of the compound was added to solutions (5.0 ml), and the suspensions were kept in an ultrasound bath for 10 min and vigorously shaken for 10 min. Suspensions were left for 30 min and filtered (MiLLex-GV, 0.22 μm). The filtered solutions were analyzed by HPLC.

5.63. Single dose rat pharmacokinetic study

Intravenous formulation of compounds **8** and **52** was prepared by dissolving in DMSO (5 mg/mL) and diluting to a final concentration of 0.5 mg/mL with 5% injectable glucose. Intravenous formulation of compound **11** was prepared by dissolving in saline (0.5 mg/mL). Compounds were intravenously administered at 0.5 mg/kg (dosing volume: 1 mL/kg) to non-fasted 8- to 10-week-old male Wistar rats (n = 2–5). 0.5 ml aliquots of blood samples were taken from the polyethylene tube cannulated in the femoral artery at 2, 5, 15, 30, 45, 60, 90, 120, and 180 min after administration, and centrifuged to obtain plasma at about 6000g for 10 min at 4 °C, which was preserved at −20 °C in a freezer. Plasma samples were extracted by solid-phase chromatography (OASIS^{TM}HLB; 60 mg/3 mL) and plasma concentrations ware determined by LC-MS/MS. Pharmacokinetic parameters ($t_{1/2}$, Cl_{tot}, $AUC_{0\text{-inf}}$ and V_{ss}) were calculated using the observed data by noncompartmental analysis (WinNonlin; Ver.3.1 Pharsight Corporation).

$t_{1/2}$: half-life; Cl_{tot}: total clearance; $AUC_{0\text{-inf}}$: area under concentration curve from hour 0 to infinity; V_{ss}: steady-state distribution volume.

5.64. Modeling

All modeling experiments were done using the program package QUANTA/CHARMm(Accelrys Inc.) on SGI workstation. Conformation of ligands and protein–ligand complexes was energy minimized using an adopted-basis Newton–Raphson method based on CHARMm force field. Docking simulations were based on crystal structure of the vitronectin receptor bound to the ligand c-RGDf(N-Me)V.[9b] In docking simulations, each ligand was manually placed in the active site based on c-RGDf(N-Me)V binding region and minimized, treating all ligand atoms plus all protein side chain within a sphere of 20 Å centered in Mn^{2+} ion as flexible.

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