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Bioorganic & Medicinal Chemistry 14 (2006) 2109–2130
Bioorganic & Medicinal Chemistry

Tricyclic pharmacophore-based molecules as novel integrin $\alpha_v\beta_3$ antagonists. Part 2: Synthesis of potent $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists

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Received 6 August 2005; revised 31 October 2005; accepted 31 October 2005
Available online 23 November 2005

Abstract—We synthesized 4-aminopiperidine derivatives of our prototype integrin $\alpha_v\beta_3$ antagonist **1** in an attempt to increase the activity and water solubility. Introduction of one or two hydrophilic moieties into the central aromatic ring and/or the benzene ring at the C-terminus of **1** increased water solubility and enhanced inhibition of cell adhesion. The results of a structure–activity relationships (SAR) study indicated that the torsion angle between the central aromatic ring and the piperidine ring, and the acidity at the sulfonamide moiety, might be important for $\alpha_v\beta_3$ receptor binding activity. Some of these compounds are novel and potent $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists with acceptable water solubility and a satisfactory early absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile.

1. Introduction

The vitronectin receptor, integrin $\alpha_v\beta_3$, is a member of the integrin superfamily of cell adhesion molecules, and is expressed in many cell types, including osteoclasts, leukocytes, vascular smooth muscle cells, and endothelial cells. Integrin $\alpha_v\beta_3$ binds a number of proteins, including vitronectin, fibrinogen, and osteopontin, through recognition of the tripeptide RGD sequence,[1] and is involved in osteoporosis, cancer growth and metastasis, diabetic retinopathy, rheumatoid arthritis, and restenosis.[2] Therefore, $\alpha_v\beta_3$ is considered as a potential drug target, and several groups are searching for orally active, small-molecular antagonists as candidate drugs to treat these chronic diseases.[3]

We are interested in integrin $\alpha_v\beta_3$ antagonists because $\alpha_v\beta_3$ is associated with adhesion and migration of vascular smooth muscle cells and leukocytes. Further, 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, has clinical efficacy in the treatment of ischemic diseases. Therefore, an injectable $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonist would be a candidate drug for acute ischemic diseases. We have already discovered highly constrained $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists possessing a tricyclic pharmacophore.[5] Although a prototype dual antagonist **1** was successfully obtained, it shows poor water solubility. Herein, we describe the synthesis of 4-aminopiperidine derivatives of **1** as novel and potent $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists exhibiting improved water solubility. We also explored the structure–activity relationships of the derivatives. The potency of these compounds is discussed in terms of not only receptor binding affinity, but also activity in the cellular context.

2. Chemistry

Antagonists possessing a piperidine tricyclic pharmacophore were generally synthesized as shown in Scheme 1. Nucleophilic substitution[6] of ethyl 4-fluorobenzoates **2a**–**e** with 4-hydroxypiperidine gave the bicyclic compounds **3a**–**e**. The alcohols **3a**–**e** were converted to amines **4a**–**e**, then introduction of a pyrimidine moiety followed by basic hydrolysis gave compounds **5a**–**e**

Keywords: Integrin $\alpha_v\beta_3$ antagonist; Integrin $\alpha_{IIb}\beta_3$ antagonist; Acute ischemic disease; 4-Aminopiperidine derivatives.
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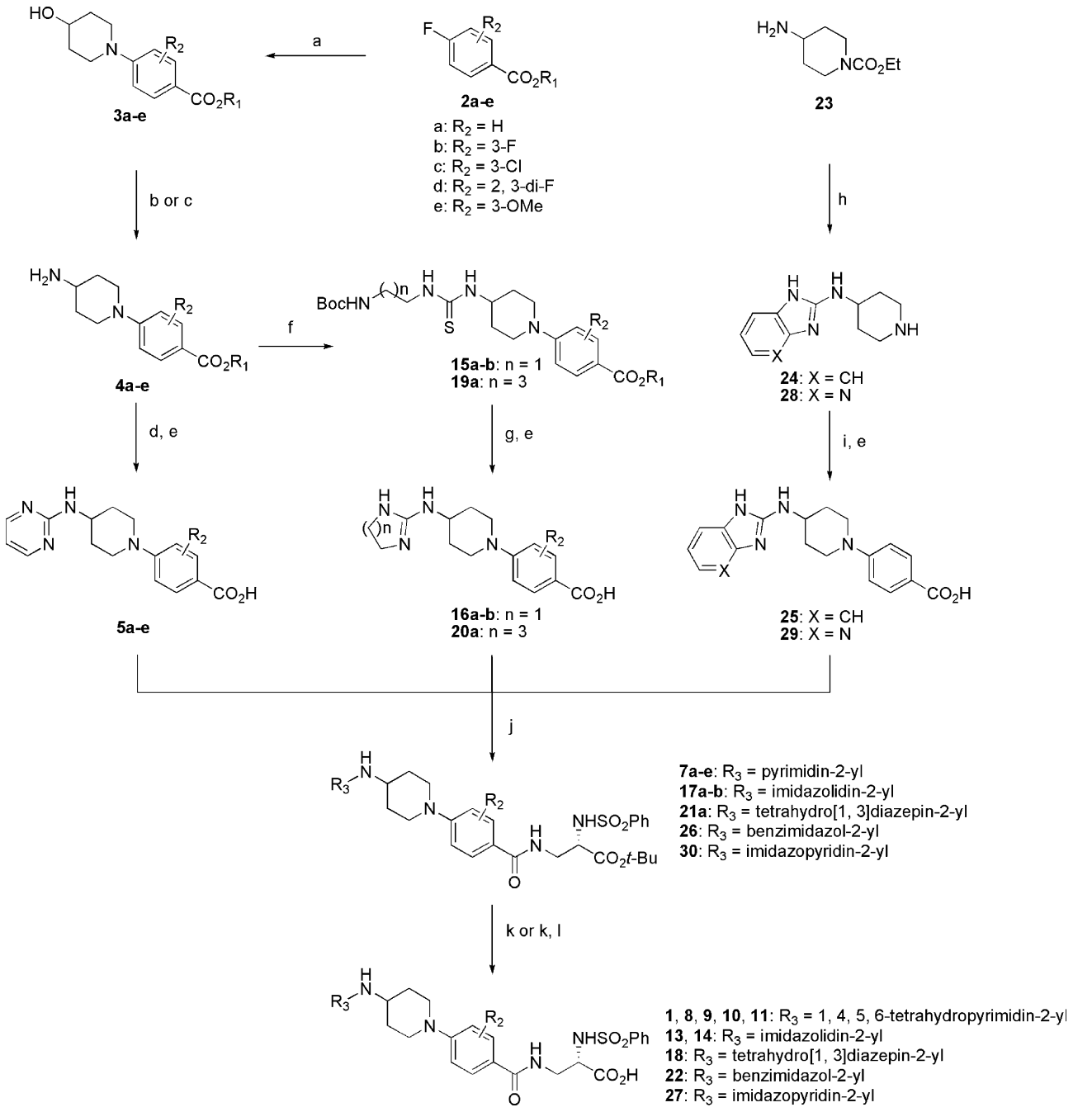
doi:10.1016/j.bmc.2005.10.061



Scheme 1. Reagents: (a) 4-hydroxypiperidine, DMSO; (b) (i) phthalimide, DEAD, THF; (ii) hydrazine; (c) (i) MsCl, Et_3N, CH_2Cl_2; (ii) NaN_3, DMF; (iii) Pd/C, H_2, dioxane; (d) 2-bromopyrimidine, *i*-Pr_2EtN, DMSO; (e) NaOH, THF, MeOH, H_2O; (f) *N*-Boc-2-isothiocyanatoethylamine, THF (for **15**), *N*-Boc-2-isothiocyanatobutylamine, THF (for **19**); (g) (i) EtBr, EtOH; (ii) TFA, H_2O; (iii) NaOEt, EtOH; (h) (i) 2-chlorobenzimidazole (for **24**), 2-chloroimidazo[4,5-*b*]pyridine (for **28**); (ii) HBr, H_2O; (i) ethyl 4-fluorobenzoate, DMSO; (j) (i) **6a**, EDC, HOBT, *N*-methylmorpholine, DMF; (k) TFA, CH_2Cl_2; (l) 10% Pd/C, H_2, dioxane, H_2O.

containing the tricyclic pharmacophore. The carboxylic acids **5a**–**e** were coupled with a diaminopropionate[7] **6a** (Fig. 1) to afford amides **7a**–**e**. Removal of the *t*-butyl group with TFA followed by hydrogenolysis of the pyrimidine ring finally gave the desired molecules **1**, **8**, **9**, **10**, and **11**. A hydroxyl derivative **12** (Table 2) was obtained from the methoxyl derivative **7e** by treatment with BBr_3. Synthesis of the antagonists **13** and **14** possessing an imidazolidinyl group required an alternative

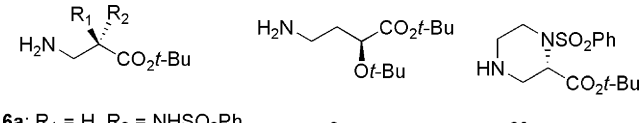
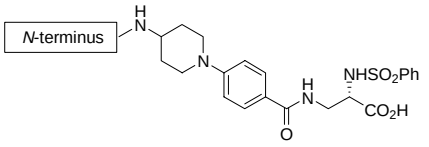
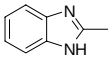
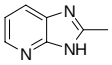


Figure 1. Chemical structures of C-termini.

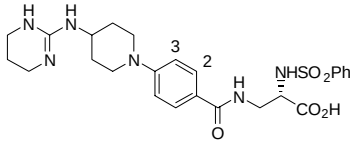
Table 1. Structure–activity relationships of the N-terminus



Compound	N-terminus	IC_{50} (nM)				$\alpha_{IIb}\beta_3/\alpha_v\beta_3$ ratio	Solubility[c]
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC[a]	hPRP[b]		
1		1.3	3.0	190	290	2.31	<1
13		0.66	0.051	52	63	0.08	1
18		0.59	0.21	79	88	0.36	NT
22		1.2	1.9	530	400	1.58	<0.5
27		0.35	0.36	770	>1000	1.03	NT
31		0.99	1.1	1300	65	1.11	1

[a] Human vascular smooth muscle cell.
[b] Human platelet aggregation.
[c] Maximum concentration as the free form in 10% aq DMSO (mg/mL).

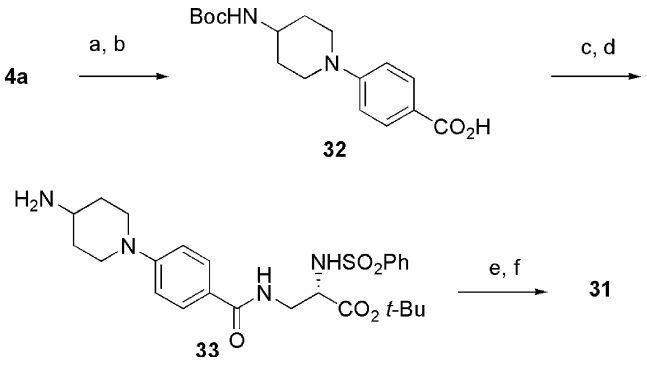
Table 2. Structure–activity relationships of the central aromatic ring



Compound	Central aromatic ring	IC_{50} (nM)				$\alpha_{IIb}\beta_3/\alpha_v\beta_3$ ratio	Solubility[a]
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC	hPRP		
1	Unsubstituted	1.3	3	190	290	2.31	<1
8	3-F	0.36	0.21	48	37	0.58	1.5
9	3-Cl	0.17	0.023	72	90	0.14	0.5
10	2, 3-Di-F	0.16	0.096	120	94	0.60	0.5
12	3-OH	0.44	0.98	530	170	2.23	>2
11	3-OMe	0.19	0.44	110	130	2.32	>2

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

method, because the coupling reaction of the amine **4a** and 2-thiomethylimidazolidine was unsuccessful. After several trials, *N*-Boc-2-isothiocyanatoethylamine[8] was found to couple with the amines **4a**,**b** to afford thioureas **15a**,**b**, which were cyclized to furnish the imidazolidines **16a**,**b**. Compound **18** possessing a 4,5,6,7-tetrahydro-1*H*-[1,3]diazepinyl group was analogously synthesized with *N*-Boc-2-isothiocyanatobutylamine. In order to synthesize benzimidazole **22**, we tried to couple 2-chlorobenzimidazole with the amine **4a**, but the reaction did not proceed. Then, 2-chlorobenzimidazole was coupled with the amine **23**, followed by deprotection to afford a tricyclic amine **24**. The amine **24** was coupled with ethyl 4-fluorobenzoate to construct the key intermediate **25**. 2-Chloroimidazo[4,5-*b*]pyridine[9] was also coupled with the amine **23** in order to prepare an azabenzimidazolyl derivative **27**. For synthesis of the guanidine **31**, the *N*-terminal functionality was introduced at the final stage of the synthetic route shown in Scheme 2. After *N*-Boc protection of the amine **4a**, hydrolysis of the ester and amidation with **6a** furnished an amide. After selective deprotection of the Boc group with HCl, the guanidine **31** was finally prepared by reaction with the pyrazole reagent[10] and then TFA.



Scheme 2. Reagents: (a) Boc_2O, NaOH, dioxane, H_2O; (b) NaOH, THF, MeOH, H_2O; (c) **6a**, EDC, HOBT, *N*-methylmorpholine, DMF; (d) HCl, MeOH; (e) 1*H*-pyrazole-1-carboxyamidine, *i*-Pr_2EtN; (f) TFA, CH_2Cl_2.

Next, the general procedure for compound **1** mentioned above was applied to modification of the C-terminal moiety. Thus, compounds **34**–**38** (Table 3) were synthesized through amide bond formation of the carboxylic acid **5** or **25** with amines **6b**–**f** (Fig. 1), respectively. The amines **6d** and **6e** possessing a hydroxyl group at the α-position were prepared through reaction with the *t*-butyl ester of L-isoserine or 4-amino-(2*S*)-hydroxybutyric acid[11] that was used in the synthesis of an MRSA-active aminoglycoside antibiotic, arbekacin.[12] Hydrogenolysis of the pyrimidine ring in acetic acid as the final step in the synthesis of compound **36** also gave **39** as a by-product. The cyclic amino ester **6f** was prepared as shown in Scheme 3. A 2-chloroethyl moiety was introduced into the primary amine **6a** and then N-trifluoroacetylation afforded the trisubstituted amine **40**. Intramolecular cyclization and deprotection of the trifluoroacetyl group finally gave the desired cyclic amine **6f**.

In order to clarify precisely the SAR at the C-terminus moiety, the convergent method was used (Scheme 4). At first, the carboxylic acid **25** and the amine **6c** were coupled to afford an amide. Introducing a Boc group onto the basic group was next planned to afford a protected compound. The Boc group was not introduced at the 4-amino group of the piperidine moiety, but at the imidazole ring, based on examination of the ^1H NMR spectrum of the product. The compound was selectively reduced to the key intermediate, the α-amine **41**, by mild hydrogenolysis using THF as a solvent. The obtained amine **41** was reacted with several sulfonyl chlorides to give derivatives **42**–**45**. Hydrogenolysis of the nitro group of **45** gave **46**. The carboxylic acid **5b** was analogously converted to an amine intermediate **47**, and the tetrahydropyrimidine derivatives **48**–**52** were similarly synthesized. The dihydroxy derivative **53** was also synthesized from the carboxylic acid **5e** using a similar methodology.

3. Results and discussion

All novel compounds were initially evaluated by means of $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ receptor binding assay. Selected compounds exhibiting strong inhibition in binding assay were further evaluated in $\alpha_v\beta_3$-mediated cell adhesion assay using VSMC (human vascular smooth muscle cell) and human vitronectin, and in hPRP (human platelet aggregation) inhibition assay, and their solubility was measured. Although an acid or a base salt shows better water solubility than its free form in general, the pH range of solubility of salts of molecules which can form zwitterions, such as our compounds, may be outside the acceptable pH range for an injectable medicine. Therefore, we initially checked the solubility of the free form in 10% aq DMSO. The aqueous solubility of several promising compounds was then evaluated quantitatively.

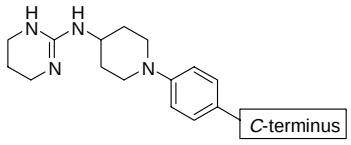
3.1. Structure–activity relationships (SAR)

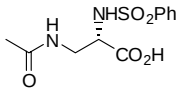
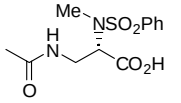
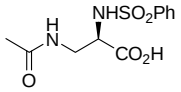
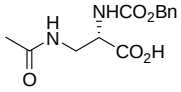
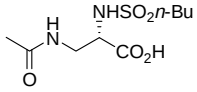
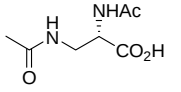
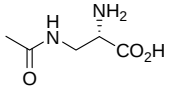
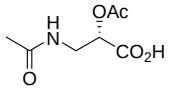
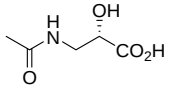
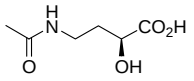
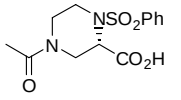
Chemical modification of the N-terminus gave important SAR data, as shown in Table 1. The cyclic guanidine analogues **1**, **13**, and **18** exhibited strong inhibitory activity towards cell adhesion. Although the imidazolidine analogue **13** showed the most potent activity and the greatest solubility among these three compounds, we selected tetrahydropyrimidine as the N-terminus for further SAR studies, because compound **1** could be synthesized in fewer steps. On the other hand, the benzimidazole **22** and the azabenzimidazole **27** exhibited only moderate activity in cell adhesion assay in spite of their strong receptor binding inhibition. Moreover, the benzimidazole **22** showed poor water solubility. The guanidine derivative **31** also exhibited only moderate activity in cell assay. Thus, appropriate lipophilicity may be required for strong inhibition of cell adhesion.

The SAR at the central aromatic ring is summarized in Table 2. In the preceding paper of this report,[5] derivatives halogenated at the aromatic C-3 position exhibited a stronger $\alpha_V\beta_3$-inhibitory activity than C-2 analogues, so we chose the C-3 position for modification. Introducing an electron-withdrawing group or an electron-donating group at the C-3 position increased the activity for receptor binding inhibition (compounds **8**, **9**, **12**, and **11**). Therefore, enhancement of activity might be explained by the steric substitution effect. The crystallographic structure of compound **8** (Fig. 2) revealed a substantial torsion angle between the central aromatic ring and the piperidine ring. Therefore, the torsion angles of various compounds substituted on the central aromatic ring might be related to the inhibitory activity on $\alpha_v\beta_3$ receptor binding. As an alternative possibility, the substituent at the 3-position might fill a cavity of the receptor site. The fluoro derivative **8** and the methoxyl derivative **11** showed not only strong inhibition of cell adhesion, but also improved solubility. Furthermore, selectivity for $\alpha_{IIb}\beta_3$ was somewhat changed by substitution at this position. The electron-withdrawing chlorine group (**9**) increased $\alpha_{IIb}\beta_3$-selectivity by approximately 10 times.

Third, the SAR of the C-terminus was investigated. The fundamental SAR of piperazine-containing molecules was disclosed in the first paper of this series.[5] (1) Modification of the amide bond decreased activity.[13] (2) A

Table 3. Structure–activity relationships of the C-terminus

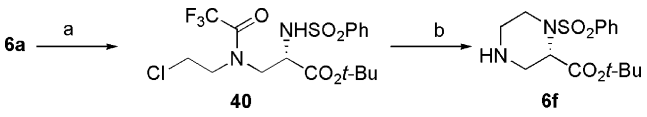


Compound	C-terminus	IC_{50} (nM)		pK_a[a]
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	
1		1.3	3.0	12.1
55		11	2.4	—
34[b]		12	3.8	12.1
35[c]		1.3	0.35	12.8
42[c]		0.53	0.59	11.6
48[b]		29	39	17.2
54[c]		280	9.1	—
39		260	430	—
36		17	9.8	15.8
37		23,000	5000	16.7
38[c]		8800	1100	—

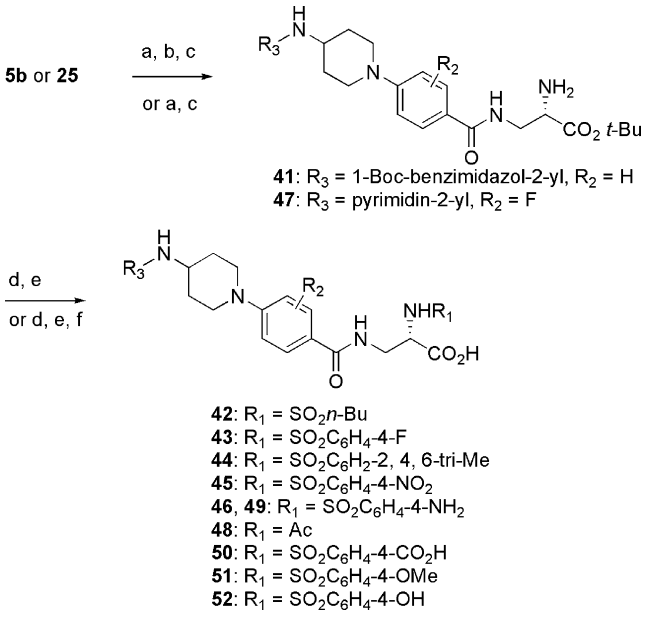
[a] Acidity at N–H or O–H.
[b] 3-Fluorobenzoyl derivative.
[c] Benzimidazole at the N-terminus.

substituent at the α-position of C-terminus was required for strong activity. Therefore, we broadly surveyed the scope of suitable α substituents, including hydrophilic functionalities. As shown in Table 3, benzenesulfonamide-containing **1** was favorable among the compounds, including **54** and **36**, with a hydrophilic functionality. Because the α-substituent has been considered as carboxylic acid-mimetic, corresponding to aspartic acid of RGD, its acidity might be important for the activity. The calculated pK_a value[14] of the α-substituent of each molecule is shown in Table 3. The results support the idea that the acidic proton of the sulfonamide is important for $\alpha_v\beta_3$-inhibitory activity. The relationships can be clearly seen by comparing **48** with **39**, and **54** with **36**, which have similar molecular size. Thus, N-methylation of the sulfonamide (**55**) dramatically decreased its activity, as expected. On the other hand, electrostatic interaction of the acidic N–H center with the exosite has been proposed in the case of $\alpha_{IIb}\beta_3$ antagonists.[15]

Because sulfonamide was considered most suitable as the α substituent, substitution of the benzenesulfonyl



Scheme 3. Reagents: (a) (i) 2-chloroacetaldehyde, $NaB(CN)H_3$, AcOH, CH_2Cl_2, MeOH; (ii) $(CF_3CO)_2O$, $NaHCO_3$, dioxane; (b) (i) DBU, DMF; (ii) concd NH_4OH, dioxane.



Scheme 4. Reagents: (a) **6c**, EDC, HOBT, *N*-methylmorpholine, DMF; (b) Boc_2O, Et_3N, CH_2Cl_2; (c) (i) Pd/C, H_2, THF; (d) R_3Cl, *i*-Pr_2EtN, DMF; (e) TFA, CH_2Cl_2; (f) 10% Pd/C, H_2, dioxane, H_2O.

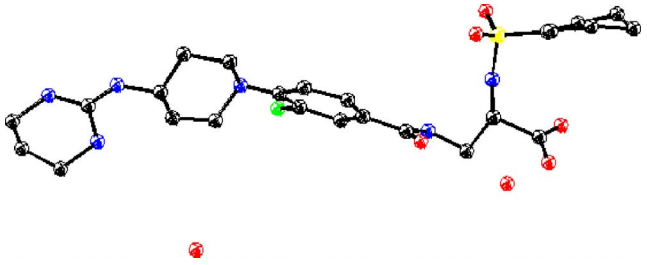


Figure 2. X-ray crystallographic data of compound **8**.

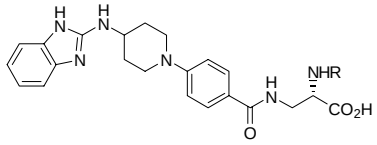
group by convergent synthesis was planned. Benzimidazole was fixed as the N-terminus because of it being convenient for facile synthesis. As shown in Table 4, compounds bearing an electron-withdrawing group (**43** or **45**) or an electron-donating group (**46**) with *para* orientation exhibited enhanced inhibitory activity on receptor binding. Replacement of the benzenesulfonyl group with a bulky substituent (**44**) also increased the activity. Next, further modifications were systematically continued (Table 5) in order to improve water solubility. Compounds having a hydrophilic functionality (an amino group **49**, a carboxyl group **50**, and a hydroxyl group **52** and **53**) showed good inhibitory activity in receptor binding assay. This result indicates that a hydrophilic functionality may be acceptable to the exosite, which has been hypothesized to be a hydrophobic binding site in the case of the $\alpha_{IIb}\beta_3$ receptor.[15] Furthermore, the solubility of these compounds was improved. However, compounds **50** and **53** showed decreased inhibitory activity on $\alpha_v\beta_3$-mediated cell adhesion. The reason why these water-soluble compounds showed weaker activity at the cellular level is not clear, but appropriate lipophilicity of the molecule might be required for interaction with cells.

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

Based on the SAR analysis, we selected compounds **8**, **11**, and **52**, possessing both strong inhibition of $\alpha_v\beta_3$-mediated cell adhesion and good water solubility (>1.5 mg/mL). The water solubility was measured quantitatively, and studies of the pharmacokinetics in rats, acute toxicity in mice, and mutagenic activity were conducted with these compounds. Water solubility was evaluated at pH 4 and pH 8. All compounds showed satisfactory pharmacokinetic parameters after intravenous infusion drug and exhibited no toxicity.[16] Compound **52** showed insufficient water solubility, because crystals were observed in water, although the other two compounds showed acceptable water solubility (Table 6).

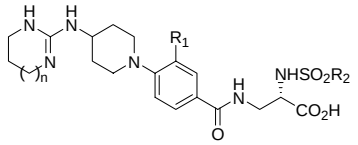
The selected $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists[17] **11** and **8** showed significant efficacy compared with a selective $\alpha_v\beta_3$ antagonist or a selective $\alpha_{IIb}\beta_3$ antagonist in a canine

Table 4. Structure–activity relationships of the sulfonamide moiety, Part 1



Compound	R	IC_{50} (nM)			
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC	hPRP
22	–SO_2Ph	1.2	1.9	530	400
43	–$SO_2C_6H_4$–4-F	0.26	0.28	300	550
44	–$SO_2C_6H_2$–2,4,6-Tri-Me	0.13	0.35	480	>1000
45	–$SO_2C_6H_4$–4-NO_2	0.35	0.60	190	>1000
46	–$SO_2C_6H_4$–4-NH_2	0.49	0.53	620	>1000

Table 5. Structure–activity relationships of the sulfonamide moiety, Part 2



Compound	R_1	n	R_2	IC_{50} (nM)				$\alpha_{IIb}\beta_3/\alpha_v\beta_3$ ratio	Solubility[a]
				$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC	hPRP		
8	F	1	–Ph	0.36	0.21	48	37	0.58	1
49	F	1	$-C_6H_4$–4-NH_2	0.29	0.087	44	110	0.30	1
50	F	1	$-C_6H_4$–4-CO_2H	0.77	1.2	660	930	1.56	<2
51	F	1	$-C_6H_4$–4-OMe	0.38	0.31	150	110	0.82	<1.5
52	F	1	$-C_6H_4$–4-OH	0.14	0.18	53	230	1.29	>2
14	F	0	–Ph	0.17	0.56	88	45	3.29	1
53	OH	1	$-C_6H_4$–4-OH	0.30	0.94	390	510	3.13	>2

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

Table 6. Quantitative water solubility and pharmacokinetics in rats[a] of selected compounds

Compound	H_2O (mg/mL)	pH 4 Mcllvaine (mg/mL)	pH 8 Mcllvaine (mg/mL)	$t_{1/2}$ (min)	CL (mL/min/kg)	AUC (μg min/mL)	V_{ss} (mL/kg)
1	<0.1						
8	0.6	0.7	0.8	26	55	9.17	510
11	1.3	4.3	2.9	46	47	10.78	559
52	0.1	0.08	0.1	30	49	10.22	689

[a] Dosed at 0.5 mg/kg iv.

acute coronary syndrome (ACS) model.[18] Furthermore, compounds **11** and **8** showed no prolongation of the bleeding time at the effective dose in canines.[19] These data indicated that the $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists **11** and **8** might be effective for treatment of reperfusion injury. Recently, an $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual inhibitor was shown to be effective against restenosis after PCTA in mice.[20] Therefore, compounds **11** and **8** might also be effective.

4. Conclusions

In summary, we identified a series of 4-aminopiperidine-based molecules as novel $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists that might be suitable for use as injectable drugs. Modifications of the prototype **1** indicated that cyclic guanidine at the N-terminus might be the most acceptable structure from the viewpoints of both cell adhesion inhibition and solubility. Introduction of one or two hydrophilic moieties at the central aromatic ring and/or benzene ring of the exosite-binding region led to improved water solubility and enhanced cell adhesion inhibition. The SAR suggested that the torsion angle between the central aromatic ring and the piperidine ring, and the acidity at the sulfonamide moiety, might be important factors for inhibitory activity on $\alpha_v\beta_3$ receptor binding. Based on evaluation of early ADMET profile and water solubility, the methoxyl derivative **11** and the fluoro derivative **8** were selected as $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists that might have utility in the treatment of acute ischemic diseases. Further studies of these compounds are ongoing.

5. Experimental

^{1}H NMR spectra were recorded on JNM-LA400 spectrometers with chemical shifts reported in ppm with internal tetramethylsilane as a standard. 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-(4-hydroxypiperidin-1-yl)benzoate (3a)

A mixture of **2a** (7.20 mL, 49.5 mmol), 4-hydroxypiperidine (5.00 g, 49.5 mmol), and DMSO (10 mL) was warmed up to 120 °C and stirred for 3 days. The cooled mixture was poured into H_2O (200 mL) with stirring vigorously. The resulting precipitate was rinsed with H_2O (50 mL) twice and hexane (50 mL) to provide **3a** (9.60 g, 38.4 mmol, 77%) as a faint yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CH_2CH_3), 1.65 (2H, m, piperidine), 1.99 (2H, br d, piperidine), 3.09 (2H, ddd, piperidine), 3.72 (2H, dt, piperidine), 3.92 (1H, tt, piperidine), 4.32 (2H, q, CH_2CH_3), 6.87 (2H, d, C_6H_4), 7.91 (2H, d, C_6H_4); EIMS *m*/*z* 249 $(M)^+$.

Using the procedures described for preparing **3a** from **2a**, the following compounds were prepared.

5.2. Methyl 3-fluoro-4-(4-hydroxypiperidin-1-yl)benzoate (3b)

The compound **2b** (33.2 g, 193 mmol) afforded **3b** (42.3 g, 167 mmol, 87%) as a faint yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74 (2H, ddt, piperidine), 2.04 (2H, m, piperidine), 2.96 (2H, ddd, piperidine), 3.50 (2H, m, piperidine), 3.89 (4H, m, CO_2Me and piperidine), 6.91 (1H, t, C_6H_3CO), 7.64 (1H, dd, C_6H_3CO), 7.72 (1H, dd, C_6H_3CO); TSPMS *m*/*z* 254 $(M+H)^+$.

5.3. Methyl 3-chloro-4-(4-hydroxypiperidin-1-yl)benzoate (3c)

The compound **2c** (6.20 g, 32.9 mmol) afforded **3c** (5.80 g, 21.6 mmol, 66%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74 (2H, ddt, piperidine), 2.04 (2H, m, piperidine), 2.89 (2H, ddd, piperidine), 3.40 (2H, m, piperidine), 3.88 (1H, m, piperidine), 3.89 (3H, s, CO_2Me), 7.02 (1H, d, C_6H_3CO), 7.85 (1H, dd, C_6H_3CO), 8.01 (1H, d, C_6H_3CO); TSPMS *m*/*z* 270 $(M+H)^+$.

5.4. Methyl 2,3-difluoro-4-(4-hydroxypiperidin-1-yl)benzoate (3d)

The compound **2d** (5.00 g, 26.3 mmol) afforded **3d** (4.73 g, 17.5 mmol, 67%) as a colorless solid, which was purified by flash column chromatography (hexane/AcOEt = 2:3): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73 (2H, m, piperidine), 2.04 (2H, m, piperidine), 3.03 (2H, ddd, piperidine), 3.54 (2H, m, piperidine), 3.90 (3H, s, Me), 3.91 (1H, m, piperidine), 6.68 (1H, ddd, Ar), 7.61 (1H, ddd, Ar); EIMS *m*/*z* 271 $(M)^+$.

5.5. Methyl 4-(4-hydroxypiperidin-1-yl)-3-methoxybenzoate (3e)

The compound **2e** (903 mg, 4.90 mmol) afforded **3e** (605 mg, 2.28 mmol, 47%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.76 (2H, ddt, piperidine), 2.04 (2H, br ddd, piperidine), 2.86 (2H, ddd, piperidine), 3.47 (2H, tt, piperidine), 3.87 (1H, m, piperidine), 3.88 (3H, s, CO_2Me), 3.92 (3H, s, C_6H_3OMe), 6.92 (1H, d, C_6H_3CO), 7.51 (1H, d, C_6H_3CO), 7.62 (1H, dd, C_6H_3CO); TSPMS *m*/*z* 266 $(M+H)^+$.

5.6. Ethyl 4-(4-aminopiperidin-1-yl)benzoate (4a)

A mixture of **3a** (2.00 g, 8.02 mmol), phthalimide (2.36 g, 16.0 mmol), and P(*n*-Bu)$_3$ (4.00 mL, 16.0 mmol) in benzene was cooled in an ice bath. To this 1,1′-(azodicarbonyl)dipiperidine (4.04 g, 16.0 mmol) was added. The cooling bath was removed and the mixture was stirred for an additional 23 h. After dilution with H_2O (300 mL), the mixture was extracted with CH_2Cl_2 three times. The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated. The resulting residue was purified by flash column chromatography ($CHCl_3$/acetone = 50:1) to give the phthalimide (1.57 g, 4.09 mmol, 51%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (3H, t, CH_2CH_3), 1.82 (2H, br d, piperidine), 2.62 (2H, dq, piperidine), 2.96 (2H, dt, piperidine), 4.01 (2H, br d, piperidine), 4.34 (2H, q, CH_2CH_3), 4.35 (1H, m, piperidine), 6.90 (2H, d, C_6H_4), 7.72 (2H, dd, phthalimide), 7.83 (2H, dd, phthalimide), 7.93 (2H, d, C_6H_4); TSPMS *m*/*z* 379 $(M+H)^+$.

To a suspension of this phthalimide (848 mg, 2.24 mmol) in MeOH (56 mL) hydrazine monohydrate (4.5 mL) was added. The mixture was stirred for 16 h at room temperature and the precipitate was filtered by glass filter. The filtrate was concentrated and the residue was purified by flash column chromatography ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) to afford **4a** (551 mg, 2.22 mmol, 99%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.35 (3H, t, CH_2CH_3), 1.43 (2H, dq, piperidine), 1.90 (2H, br d, piperidine), 2.84 (1H, m, piperidine), 2.89 (2H, br t, piperidine), 3.92 (2H, br d, piperidine), 4.28 (2H, q, CH_2CH_3), 6.94 (2H, d, C_6H_4), 7.84 (2H, d, C_6H_4); TSPMS *m*/*z* 249 $(M+H)^+$.

5.7. Methyl 4-(4-aminopiperidin-1-yl)-3-fluorobenzoate (4b)

To a mixture of **3b** (24.8 g, 97.8 mmol), Et_3N (70.0 mL, 502 mmol), and CH_2Cl_2 (700 mL) MsCl (11.5 mL, 149 mmol) was added dropwise. The mixture was stirred for an additional 1 h. The mixture was poured into water (1000 mL) and $CHCl_3$, and extracted with $CHCl_3$ twice. The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was added water (500 mL) and extracted with organic layers (hexane/AcOEt/CH_2Cl_2 = 1:1:1) (1 L) twice. The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated to provide methyl 3-fluoro-4-{4-(methanesulfonyloxy)piperidin-1-yl}benzoate (29.9 g, 90.2 mmol, 92%).

To this (10.0 g, 27.6 mmol) DMF (50 mL) and NaN_3 (3.87 g, 59.5 mmol) were added. The mixture was heated at 80 °C for 5 h. The cooled mixture was poured into water (1 L) and extracted with AcOEt twice. The combined organic layer was washed with brine (500 mL) twice, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was added water (1 L) and extracted with hexane twice. The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated to provide methyl 4-(4-azidopiperidin-1-yl)-3-fluorobenzoate (7.75 g, 27.8 mmol, 100%) as a brown syrup: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82 (2H, ddt, piperidine), 2.05 (2H, m, piperidine), 2.99 (2H, ddd, piperidine), 3.47 (2H, m, piperidine), 3.62 (1H, tt, piperidine), 3.88 (3H, s, CO_2Me), 6.92 (1H, t, C_6H_3CO), 7.66 (1H, dd, C_6H_3CO), 7.74 (1H, dd, C_6H_3CO); TSPMS *m*/*z* 279 $(M+H)^+$.

To a solution of the azide (7.50 g, 26.9 mmol), 1,4-dioxane (190 mL), AcOH (27 mL), and water (54 mL) 10% Pd/C (750 mg) was added and the mixture was hydrogenated under H_2 for 4 h at room temperature. The mixture was filtered through Celite, and solids were washed with MeOH. The filtrate was concentrated. The residue was dissolved in 0.5 M HCl (1 L) and washed with AcOEt (500 mL) twice. The aqueous layer was cooled to 0 °C, alkalized to pH 14 using concd NH_4OH, warmed to room temperature, saturated with

NaCl, and extracted with CH_2Cl_2 (1 L) three times. The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to provide **4b** (5.78 g, 22.9 mmol, 85%) as a faint yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (2H, dq, piperidine), 1.94 (2H, br d, piperidine), 2.85 (3H, m, piperidine), 3.58 (2H, dt, piperidine), 3.88 (3H, s, CO_2Me), 6.92 (1H, t, C_6H_3CO), 7.65 (1H, dd, C_6H_3CO), 7.73 (1H, dd, C_6H_3CO); TSPMS 253 $(M+H)^+$.

Using the procedures described for preparing **4b** from **3b**, the following compounds were prepared.

5.8. Methyl 4-(4-aminopiperidin-1-yl)-3-chlorobenzoate (4c)

The compound **3c** afforded **4c** (595 mg, 2.22 mmol, 10% in three steps) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.84 (2H, dq, piperidine), 2.12 (2H, m, piperidine), 2.86 (2H, dt, piperidine), 3.28 (1H, m, piperidine), 3.56 (2H, br d, piperidine), 3.88 (3H, S, CO_2Me), 7.19 (1H, d, C_6H_3CO), 7.90 (1H, dd, C_6H_3CO), 7.98 (1H, d, C_6H_3CO); TSPMS *m/z* 269 $(M+H)^+$.

5.9. Methyl 4-(4-aminopiperidin-1-yl)-2,3-difluorobenzoate (4d)

The alcohol **3d** afforded **4d** (2.10 g, 7.78 mmol, 49% in three steps) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (2H, m, piperidine), 1.93 (2H, m, piperidine), 2.83–2.93 (3H, m, piperidine), 3.62 (2H, br d, piperidine), 3.90 (3H, s, Me), 6.66 (1H, ddd, Ar), 7.60 (1H, ddd, Ar); ESIMS *m/z* 271 $(M+H)^+$.

5.10. Methyl 4-(4-aminopiperidin-1-yl)-3-methoxybenzoate (4e)

The alcohol **3e** afforded **4e** as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (2H, dq, piperidine), 1.95 (2H, br d, piperidine), 2.70 (2H, dt, piperidine), 2.82 (1H, tt, piperidine), 3.57 (2H, br d, piperidine), 3.88 (3H, s, OMe), 3.92 (3H, s, OMe), 6.91 (1H, d, C_6H_3), 7.50 (1H, d, C_6H_3), 7.65 (1H, dd, C_6H_3).

5.11. 4-{4-(Pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (5a)

Step 1: To a solution of **4a** (250 mg, 1.01 mmol) in DMF (10 mL) 2-bromopyrimidine (240 mg, 1.51 mmol) and *i*-Pr_2NEt (0.90 mL, 5.05 mmol) were added. The reaction mixture was heated at 125 °C for 10 h. The cooled mixture was added CH_2Cl_2 and brine, and extracted three times. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated. The resulting residue was purified by silica gel flash column chromatography (hexane/AcOEt = 1:1) to give ethyl 4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (212 mg, 0.649 mmol, 64%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CH_2CH_3), 1.61 (2H, br q, piperidine), 2.17 (2H, br d, piperidine), 3.08 (2H, br t, piperidine), 3.84 (2H, br d, piperidine), 4.06 (1H, m, piperidine), 4.33 (2H, q, CH_2CH_3), 6.55 (1H, t, pyrimidine), 6.89 (2H, d, C_6H_4), 7.92 (2H, d, C_6H_4), 8.28 (2H, d, pyrimidine); EIMS *m/z* 326 $(M)^+$.

Step 2: To a solution of this benzoate (100 mg, 0.306 mmol) in a mixture of THF (9.0 mL) and MeOH (3.0 mL) 1 M NaOH (3.0 mL) was added. The reaction mixture was stirred for 8 h at 40 °C, and concentrated. The residue was purified using silica gel flash column chromatography ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) to give **5a** (75.0 mg, 0.251 mmol, 82%) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.53 (2H, br q, piperidine), 1.91 (2H, br d, piperidine), 2.88 (2H, br t, piperidine), 3.82 (2H, br d, piperidine), 3.90 (1H, m, piperidine), 6.54 (1H, t, pyrimidine), 6.89 (2H, br d, C_6H_4), 7.73 (2H, br d, C_6H_4), 8.25 (2H, d, pyrimidine); EIMS *m/z* 298 $(M)^+$.

Using the procedures described for preparing **5a** from **4a**, the following compounds were prepared.

5.12. 3-Fluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (5b)

The amine **4b** (5.78 g, 22.9 mmol) afforded **5b** (4.96 g, 15.7 mmol, 78% in two steps) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.63 (2H, br q, piperidine), 1.96 (2H, br d, piperidine), 2.88 (2H, br t, piperidine), 3.53 (2H, br d, piperidine), 3.89 (1H, m, piperidine), 6.54 (1H, t, pyrimidine), 7.08 (1H, t, C_6H_3CO), 7.54 (1H, dd, C_6H_3CO), 7.66 (1H, dd, C_6H_3CO), 8.26 (2H, d, pyrimidine); TSPMS *m/z* 317 $(M+H)^+$.

5.13. 3-Chloro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (5c)

The amine **4c** afforded **5c** (109 mg, 0.328 mmol, 34% in two steps) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.67 (2H, dq, piperidine), 1.97 (2H, m, piperidine), 2.81 (2H, br t, piperidine), 3.41 (2H, br d, piperidine), 3.89 (1H, br d, piperidine), 6.54 (1H, t, pyrimidine), 7.19 (1H, t, C_6H_3CO), 7.81 (1H, dd, C_6H_3CO), 7.85 (1H, d, C_6H_3CO), 8.26 (2H, d, pyrimidine); FABMS *m/z* 333 $(M+H)^+$.

5.14. 2,3-Difluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (5d)

The amine **4d** afforded **5d** (402 mg, 1.20 mmol, 44% in two steps) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.63 (2H, m, piperidine), 1.97 (2H, br d, piperidine), 2.97 (2H, br t, piperidine), 3.59 (2H, br d, piperidine), 3.92 (1H, m, piperidine), 6.56 (1H, t, pyrimidine), 6.90 (1H, br t, Ar), 7.58 (1H, br t, Ar), 8.28 (2H, d, pyrimidine); ESIMS *m/z* 335 $(M+H)^+$.

5.15. 3-Methoxy-4-{4-(pyrimidin-2-ylamino)-piperidin-1-yl}benzoic acid (5e)

The amine **4e** afforded **5e** (261 mg, 0.796 mmol, 44% from **3e**) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.64 (2H, dq, piperidine), 1.93 (2H, br d, piperidine), 2.70 (2H, br t, piperidine), 3.50 (2H, br d, piperidine), 3.83 (3H, s, C_6H_3OMe), 3.85 (1H, m, piperidine), 6.55 (1H, t, pyrimidine), 6.93 (1H, d, C_6H_3CO), 7.41 (1H, d, C_6H_3CO), 7.49 (1H, dd, C_6H_3CO), 8.27 (2H, d, pyrimidine); ESIMS *m/z* 329 $(M+H)^+$.

5.16. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (7a)

The carboxylic acid **5a** (6.00 mg, 0.0201 mmol), *i*-Pr_2NEt (5.3 μL, 0.0302 mmol), and (benzotriazol-1-yloxy)tris (dimethylamino)phosphonium hexafluorophosphate (BOP) (13.3 mg, 0.0302 mmol) were dissolved in a mixture of CH_2Cl_2 (1.0 mL) and DMF (1.0 mL), and stirred for 3 h at room temperature. To the mixture, a solution of hydrochloride salt of *t*-butyl (2*S*)-*N*-benzenesulfonyl-2,3-diaminopropionate **6a**[7] (8.10 mg, 0.0241 mmol) in CH_2Cl_2 (1.0 mL) was added at −10 °C. The solution was added *i*-Pr_2NEt (5.3 μL, 0.0302 mmol) and stirred an additional 2 h at the same temperature. The solvent was removed in vacuo and the residue was purified by silica gel preparative TLC ($CHCl_3$/MeOH = 10:1) to give **7a** (7.00 mg, 0.0121 mmol, 60%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (9H, s, *t*-Bu), 1.60 (2H, m, piperidine), 2.18 (2H, br d, piperidine), 3.06 (2H, br t, piperidine), 3.57 (1H, ddd, CONHCH_2CH), 3.81 (2H, br d, piperidine), 3.90 (2H, m, CONHCH_2C*H*), 4.05 (1H, m, piperidine), 6.55 (1H, t, pyrimidine), 6.91 (2H, d, C_6H_4), 7.49 (2H, m, C_6H_5), 7.57 (1H, m, C_6H_5), 7.70 (2H, d, C_6H_4), 7.86 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 581 $(M+H)^+$; $[\alpha]_D^{25}$ +24° (*c* 0.35, MeOH).

5.17. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[3-fluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (7b)

Hydrochloride salts of the propionate **6a** (1.17 g, 3.48 mmol) and **5b** (1.00 g, 3.16 mmol) were suspended in DMF (30 mL). HOBt (512 mg, 3.79 mmol), NMM (1.04 mL, 9.48 mmol), and EDC (727 mg, 3.79 mmol) were added and the reaction mixture was stirred for 13 h. The mixture was added a mixture of saturated solution of $NaHCO_3$ (300 mL) and aqueous K_2CO_3 (100 mL) and extracted with CH_2Cl_2 (200 mL) three times. The organic layer was washed with brine (500 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 25:1) to give **7b** (1.77 g, 2.95 mmol, 93%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (9H, s, *t*-Bu), 1.74 (2H, br q, piperidine), 2.12 (2H, br d, piperidine), 2.94 (2H, br t, piperidine), 3.49 (1H, dd, CONHCH_2CH), 3.59 (2H, br d, piperidine), 3.66 (1H, dd, CONHCH_2CH), 3.95 (1H, m, piperidine), 4.12 (1H, br t, CONHCH$_2$C*H*), 6.59 (1H, t, pyrimidine), 7.08 (1H, t, C_6H_3CO), 7.47 (3H, m, C_6H_3CO and C_6H_5), 7.54 (2H, m, C_6H_3CO and C_6H_5), 7.83 (2H, d, C_6H_5), 8.27 (2H, d, pyrimidine); TSPMS *m*/*z* 599 $(M+H)^+$; $[\alpha]_D^{25}$ +26° (*c* 0.52, MeOH).

Using the procedures described for preparing **7b** from **5b**, the following compounds were prepared.

5.18. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[3-chloro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (7c)

The carboxylic acid **5c** (107 mg, 0.321 mmol) afforded **7c** (168 mg, 0.274 mmol, 85%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (9H, s, *t*-Bu), 1.75 (2H, br q, piperidine), 2.21 (2H, br d, piperidine), 2.91 (2H, br t, piperidine), 3.47 (2H, br d, piperidine), 3.56 (1H, ddd, CONHCH_2CH), 3.90 (2H, m, CONHCH_2CH), 4.03 (1H, m, piperidine), 6. 55 (1H, t, pyrimidine), 7.07 (1H, d, C_6H_3CO), 7.51 (2H, m, C_6H_5), 7.58 (1H, m, C_6H_5), 7.65 (1H, dd, C_6H_3CO), 7.84 (1H, dd, C_6H_3CO), 7.86 (2H, br d, C_6H_5), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 615 $(M+H)^+$; $[\alpha]_D^{25}$ +49° (*c* 1.0, CH_2Cl_2).

5.19. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[2,3-difluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (7d)

The carboxylic acid **5d** (130 mg, 0.389 mmol) afforded **7d** (239 mg, 0.388 mmol, 100%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (9H, s, *t*-Bu), 1.70 (2H, br q, piperidine), 2.19 (2H, br s, piperidine), 3.01 (2H, br t, piperidine), 3.59 (2H, br d, piperidine), 3.67–3.86 (2H, m, CONHCH_2CH), 3.97–4.07 (2H, m, CONHCH_2CH and piperidine), 6.54 (1H, t, pyrimidine), 6.74 (1H, br t, $C_6H_2F_2$), 7.46 (2H, br t, C_6H_5), 7.53 (1H, br t, C_6H_5), 7.68 (1H, br t, $C_6H_2F_2$), 7.85 (2H, m, C_6H_5), 8.30 (2H, d, pyrimidine); ESIMS *m*/*z* 617 $(M+H)^+$; $[\alpha]_D^{25}$ +39° (*c* 1.0, $CHCl_3$).

5.20. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[3-methoxy-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (7e)

The carboxylic acid **5e** (50.0 mg, 0.152 mmol) afforded **7e** (92.5 mg, 0.152 mmol, 100%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (9H, s, *t*-Bu), 1.76 (2H, br dq, piperidine), 2.20 (2H, br d, piperidine), 2.85 (2H, br t, piperidine), 3.53 (3H, m, piperidine and CONHCH_2CH), 3. 93 (1H, m, CONHCH_2CH), 3.95 (3H, s, C_6H_3OMe), 4.02 (1H, m, piperidine), 6.54 (1H, t, pyrimidine), 6.95 (1H, d, C_6H_3CO), 7.33 (1H, dd, C_6H_3CO), 7.41 (1H, d, C_6H_3CO), 7.50 (2H, br t, C_6H_5), 7. 58 (1H, br t, C_6H_5), 7.86 (2H, br d, C_6H_5), 8.29 (2H, d, pyrimidine); FABMS *m*/*z* 611 $(M+H)^+$; $[\alpha]_D^{22}$ +22° (*c* 1.0, MeOH).

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

Step 1: The compound **7a** (7.00 mg, 0.0121 mmol) was dissolved in CH_2Cl_2 (0.3 mL) and cooled in an ice bath, to which TFA (0.3 mL) was added. The mixture was stirred at room temperature for 2 h. The solvent was evaporated in vacuo and the residue was purified by silica gel preparative TLC ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1), and then LH-20 (MeOH) to give (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (6.30 mg, 0.0120 mmol, 99%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.56 (2H, dq, piperidine), 1.99 (2H, br d, piperidine), 2.90 (2H, br t, piperidine), 3.46 (1H, dd, CONHCH_2CH), 3.56 (1H, dd, CONHCH_2CH), 3.63 (1H, dd, CONHCH$_2$C*H*), 3.80 (2H, br d, piperidine), 3.89 (1H, m, piperidine), 6.49 (1H, t, pyrimidine), 6.89 (2H, d, C_6H_4), 7.36 (2H, m, C_6H_5), 7.43 (1H, m, C_6H_5), 7.60

(2H, d, C_6H_4), 7.75 (2H, m, C_6H_5), 8.16 (2H, d, pyrimidine); FABMS *m/z* 525 $(M+H)^+$; $[\alpha]_D^{23}$ +65° (*c* 0.36, MeOH).

Step 2: To a solution of the above compound (3.60 mg, 0.00686 mmol), concd HCl (0.5 mL), and AcOH (5.0 mL), 10% Pd/C (1.8 mg) was added and the mixture was hydrogenated under H_2 (50 psi) in a Parr shaker for 2.5 h at room temperature. The mixture was filtered through Celite, and solids were washed with MeOH twice and H_2O twice. The filtrate was concentrated. Toluene was added and the mixture was again concentrated in vacuo. The residue was purified by silica gel preparative TLC (CH_2Cl_2/EtOH/concd NH_4OH/H_2O = 8:8:1:1), and then LH-20 (MeOH) to yield **1** (3.20 mg, 0.00605 mmol, 88%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.49 (2H, br q, piperidine), 1.85 (4H, m, piperidine and tetrahydropyrimidine), 2.84 (2H, br t, piperidine), 3.26 (4H, t, tetrahydropyrimidine), 3.45 (2H, m, CONHCH_2CH and piperidine), 3.53 (1H, dd, CONHCH_2CH), 3.63 (1H, dd, $CONHCH_2CH$), 3.73 (2H, br d, piperidine), 6.85 (2H, d, C_6H_4), 7.37 (2H, m, C_6H_5), 7.44 (1H, m, C_6H_5), 7.59 (2H, d, C_6H_4), 7.75 (2H, m, C_6H_5); FABMS *m/z* 529 $(M+H)^+$; $[\alpha]_D^{20}$ +69° (*c* 0.16, MeOH).

5.22. (2*S*)-Benzenesulfonylamino-3-[3-fluoro-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (8)

Step 1: The *t*-butyl ester **7b** (600 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (10 mL), added TFA (10 mL), and stirred at room temperature for 4 h. The solvent was evaporated in vacuo to give the TFA salt of (2*S*)-benzenesulfonylamino-3-[3-fluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid as a colorless solid.

Step 2: To a solution of TFA salt of this compound, 1,4-dioxane (10 mL), and H_2O (1.0 mL) 10% Pd/C (120 mg) were added, and the mixture was hydrogenated under H_2 for 24 h at room temperature. The mixture was filtered through Celite, and solids were washed with 1,4-dioxane/H_2O (10:1). The filtrate was concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/EtOH/concd NH_4OH/ H_2O = 8:8:1:1), and then LH-20 (MeOH) to give **8** (451 mg, 0.820 mmol, 82% in two steps) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.68 (2H, br ddd, *J* = 11.8 Hz, 11.8 Hz, 12.7 Hz, piperidine), 1.96 (2H, quintet, *J* = 5.8 Hz, tetrahydropyrimidine), 2.02 (2H, br d, *J* = 12.7 Hz, piperidine), 2.87 (2H, br dd, *J* = 11.8 Hz, 12.3 Hz, piperidine), 3.37 (4H, t, *J* = 5.8 Hz, tetrahydropyrimidine), 3.48 (1H, m, piperidine), 3.52 (2H, br d, *J* = 12.3 Hz, piperidine), 3.57 (1H, dd, *J* = 7.6 Hz, 13.9 Hz, $CONHCH_2CH$), 3.65 (1H, dd, *J* = 5.1 Hz, 13.9 Hz, $CONHCH_2CH$), 3.73 (1H, dd, *J* = 5.1 Hz, 7.6 Hz, $CONHCH_2CH$), 7.04 (1H, t, *J* = 8.6 Hz, C_6H_3CO), 7.53 (5H, m, C_6H_3CO and C_6H_5), 7.86 (2H, br d, *J* = 7.3 Hz, C_6H_5); ^{13}C NMR (DMSO-d_6) δ 19.8, 31.7, 31.8, 37.8, 42.6, 47.1, 48.9, 55.4, 114.5 (d, J_{CF} = 21.5 Hz), 118.4 (d, J_{CF} = 2.5 Hz), 123.6, 126.6, 127.9 (d, J_{CF} = 6.6 Hz), 128.9, 132.2, 140.8, 142.2 (d, J_{CF} = 8.3 Hz), 152.1, 153.8 (d, J_{CF} = 245.6 Hz), 164.2, 173.4; TSPMS *m/z* 547 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}FN_6O_5S$: 547.2139, found: 547.2148; $[\alpha]_D^{26}$ +79° (*c* 0.56, DMSO).

Using the procedures described for preparing **8** from **7b**, the following compounds were prepared.

5.23. (2*S*)-Benzenesulfonylamino-3-[3-chloro-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (9)

The compound **7c** afforded **9** (15.5 mg, 0.0267 mmol, 36% in two steps) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.73 (2H, m, piperidine), 1.97 (2H, quintet, tetrahydropyrimidine), 2.04 (2H, m, piperidine), 2.84 (2H, br t, piperidine), 3.37 (4H, br t, tetrahydropyrimidine), 3.43 (2H, br d, piperidine), 3.48 (1H, m, piperidine), 3.56 (1H, dd, CONHCH_2CH), 3.66 (1H, dd, CONHCH_2CH), 3.74 (1H, dd, $CONHCH_2CH$), 7.15 (1H, d, pyrimidine), 7.47 (2H, m, C_6H_5), 7.53 (1H, m, C_6H_5), 7.72 (1H, dd, C_6H_3CO), 7.85 (3H, m, C_6H_3CO and C_6H_5); TSPMS *m/z* 563 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}ClN_6O_5S$: 563.1843, found: 563.1830; $[\alpha]_D^{25}$ +64° (*c* 0.20, MeOH).

5.24. (2*S*)-Benzenesulfonylamino-3-[2,3-difluoro-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (10)

The compound **7d** afforded **10** (87.2 mg, 0.155 mmol, 77% in two steps) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54 (2H, m, piperidine), 1.81 (2H, quintet, tetrahydropyrimidine), 1.87 (2H, br d, piperidine), 2.73 (2H, br t, piperidine), 3.08 (1H, br t, CONHCH_2CH), 3.21 (4H, br t, tetrahydropyrimidine), 3.43 (2H, m, piperidine), 3.80 (1H, m, CONHCH_2CH), 6.87 (1H, br t, $C_6H_2F_2$), 7.51 (1H, br t, $C_6H_2F_2$), 7.56 (2H, m, C_6H_5), 7.63 (1H, m, C_6H_5), 7.84 (2H, m, C_6H_5); ESIMS *m/z* 565 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{30}F_2N_6O_5S$: 565.2045, found: 565.2033; $[\alpha]_D^{25}$ +92° (*c* 0.60, DMSO).

5.25. (2*S*)-Benzenesulfonylamino-3-[3-methoxy-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (11)

The compound **7e** afforded **11** (16.6 mg, 0.0297 mmol, 63% in two steps) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.39–1.43 (2H, m, piperidine), 1.73–1.80 (4H, m, piperidine and tetrahydropyrimidine), 2.39–2.47 (2H, m, piperidine), 3.08–3.15 (2H, m, CONHCH_2CH and piperidine), 3.19 (4H, m, tetrahydropyrimidine), 3.28 (2H, m, piperidine), 3.48 (1H, m, $CONHCH_2CH$), 3.60 (1H, m, CONHCH_2CH), 3.68 (3H, s, C_6H_3OMe), 6.77 (1H, d, *J* = 8.7 Hz, C_6H_3CO), 7.26 (1H, d, *J* = 8.7 Hz, C_6H_3CO), 7.28 (1H, s, C_6H_3CO), 7.53–7.57 (2H, m, C_6H_5), 7.61 (1H, m, C_6H_5), 7.83 (1H, d, *J* = 6.8 Hz, C_6H_5), 7.84 (1H, d, *J* = 6.8 Hz, C_6H_5), 8.31 (1H, br t, *J* = 5.0 Hz, CONHCH_2CH), 8.71 (1H, br s, NH), 9.42 (1H, br d, *J* = 7.6 Hz, NH); ^{3}C NMR (DMSO-d_6) 19.8, 31.8, 32.1, 37.8, 42.6, 47.3, 48.9, 49.0, 55.3, 55.4, 110.5, 117.2, 119.6, 126.6, 128.3, 129.0, 132.3, 140.8, 143.9,

151.3, 152.0, 165.4, 173.5; FABMS *m/z* 559 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{26}H_{34}N_6O_6S$: 559.2339, found: 599.2343; $[\alpha]_D^{25}$ +76° (*c* 0.83, MeOH/concd NH_4OH (10:1)).

5.26. (2*S*)-Benzenesulfonylamino-3-[3-hydroxy-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (12)

To a solution of **7e** (23.7 mg, 0.0388 mmol) and dichloroethane (3.6 mL) BBr_3 (1.0 M in CH_2Cl_2, 0.70 mL, 0.700 mmol) was added. The mixture was warmed to 40 °C and stirred for 7 h. A mixture of 1,4-dioxane (1.0 mL), water (0.1 mL), and NH_4OH (1.0 mL) was added to the mixture. The concentrated residue was added water (20 mL) and washed with CH_2Cl_2 twice. The aqueous layer was evaporated and the residue was purified by CHP-20P (Mitsubishi Chemical Corporation) (MeOH:water = 3:7) to prepare (2*S*)-benzenesulfonylamino-3-[3-hydroxy-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (8.40 mg, 0.0155 mmol, 40%) as a colorless solid: ^1H NMR (400 MHz, CD_3OD) δ 1.79 (2H, dq, piperidine), 2.10 (2H, br d, piperidine), 2.81 (2H, br t, piperidine), 3.46 (2H, br d, piperidine), 3.54 (1H, dd, CONHCH_2CH), 3.66 (1H, dd, CONHCH_2CH), 3.77 (1H, dd, CONHCH$_2$CH), 3.93 (1H, m, piperidine), 6.59 (1H, t, pyrimidine), 7.05 (1H, d, C_6H_3CO), 7.27 (2H, m, C_6H_3CO), 7.45 (2H, br t, C_6H_5), 7.52 (1H, br t, C_6H_5), 7.85 (2H, br d, C_6H_5), 8.27 (2H, d, pyrimidine); APCIMS *m/z* 541 $(M+H)^+$; $[\alpha]_D^{26}$ +22° (*c* 0.42, MeOH).

The title compound **12** (3.50 mg, 0.00643 mmol, 43%) was synthesized from this compound following the general procedure for **8** (Step 2): ^1H NMR (400 MHz, CD_3OD) δ 1.74 (2H, br dq, piperidine), 1.99 (4H, m, piperidine and tetrahydropyrimidine), 2.74 (2H, br t, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.44 (3H, m, piperidine), 3.56 (1H, dd, CONHCH_2CH), 3.64 (1H, dd, CONHCH_2CH), 3.74 (1H, dd, CONHCH$_2$CH), 6.98 (1H, d, C_6H_3CO), 7.27 (2H, m, C_6H_3CO), 7.47 (2H, br t, C_6H_5), 7.54 (1H, br t, C_6H_5), 7.86 (2H, br d, C_6H_5); ESIMS *m/z* 545 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_6S$: 545.2182, found: 545.2178; $[\alpha]_D^{23}$ +40° (*c* 0.18, MeOH).

5.27. Ethyl 4-[4-[3-{2-(*t*-butoxycarbonylamino)ethyl}thioureido]piperidin-1-yl]benzoate (15a)

N-Boc-2-isothiocyanatoethylamine (29.0 mg, 0.145 mmol) was added to a solution of **4a** (30.0 mg, 0.121 mmol) in THF (0.5 mL). The solution was stirred for 19 h under argon. The solvent was removed in vacuo. The product was purified by silica gel preparative TLC (CH_2Cl_2/MeOH = 7:1), and then LH-20 (MeOH) to yield **15a** (50.1 mg, 0.111 mmol, 92%) as a colorless solid: ^1H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CO_2Et), 1.42 (9H, s, *t*-Bu), 1.62 (2H, m, piperidine), 2.15 (2H, m, piperidine), 3.03 (2H, br t, piperidine), 3.31 (2H, m, CH_2CH_2), 3.55 (2H, m, CH_2CH_2), 3.85 (2H, br d, piperidine), 4.33 (2H, q, CO_2Et), 4.98 (1H, m, piperidine), 6.97 (2H, d, C_6H_4CO), 7.92 (2H, d, C_6H_4CO); TSPMS *m/z* 451 $(M+H)^+$.

5.28. Ethyl 4-[4-[3-{2-(*t*-butoxycarbonylamino)ethyl}thioureido]piperidin-1-yl]-3-fluorobenzoate (15b)

The title compound was synthesized from **4b** following the general procedure for **15a**: FABMS *m/z* 455 $(M+H)^+$.

5.29. 4-{4-(4,5-Dihydro-1*H*-imidazol-2-ylamino)piperidin-1-yl}benzoic acid (16a)

Compound **15a** (14.2 mg, 0.0315 mmol) was dissolved in a solution of bromoethane (0.5 mL) and EtOH (0.5 mL). The mixture was heated at reflux for 13 h under argon. The solvent was removed in vacuo. The residue was purified by silica gel preparative TLC (CH_2Cl_2/MeOH = 7:1) to afford the crude compound.

The resulting compound was dissolved in TFA (1.0 mL) and water (0.5 mL), and stirred at room temperature for 3 h. The residue after concentration was dissolved in EtOH (1.0 mL) and added dropwise over 3 h to a solution of sodium ethoxide (18.0 mg, 0.261 mmol) in EtOH (1.0 mL). The system was stirred under argon for an additional 16 h, at which point the solvent was removed in vacuo. The residue was purified by silica gel preparative TLC (CH_2Cl_2/MeOH = 7:1) to yield ethyl 4-{4-(4,5-dihydro-1*H*-imidazol-2-ylamino)piperidin-1-yl}benzoate (6.10 mg, 0.0193 mmol, 61% from **15a**) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, CO_2Et), 1.76 (2H, br q, piperidine), 2.02 (2H, br d, piperidine), 2.95 (2H, ddd, piperidine), 3.53 (1H, m, piperidine), 3.70 (4H, s, CH_2CH_2), 3.76 (2H, br d, piperidine), 4.32 (2H, q, CO_2Et), 6.81 (2H, d, C_6H_4CO), 7.89 (2H, d, C_6H_4CO); TSPMS *m/z* 317 $(M+H)^+$.

The ester (1.27 g, 4.00 mmol) afforded **16a** (1.15 g, 4.00 mmol, 100%) as a colorless solid following the general procedure for **5a** (Step 2): ^1H NMR (400 MHz, CD_3OD) δ 1.64 (2H, dq, piperidine), 2.03 (2H, br d, piperidine), 2.96 (2H, br t, piperidine), 3.53 (1H, m, piperidine), 3.72 (4H, s, CH_2CH_2), 3.88 (2H, br d, piperidine), 6.95 (2H, d, C_6H_4CO), 7.86 (2H, d, C_6H_4CO); FABMS *m/z* 289 $(M+H)^+$.

5.30. 3-Fluoro-4-{4-(4,5-dihydro-1*H*-imidazol-2-ylamino)piperidin-1-yl}benzoic acid (16b)

The title compound (49.0 mg, 0.153 mmol, 74% from **4b**) was synthesized from **15b** and *N*-Boc-4-isothiocyanatobutylamine following the general procedure for **16a**: ^1H NMR (400 MHz, CD_3OD) δ 1.74 (2H, br dq, piperidine), 2.06 (2H, br d, piperidine), 2.92 (2H, br t, piperidine), 3.51 (1H, m, piperidine), 3.61 (2H, br d, piperidine), 3.73 (4H, s, CH_2CH_2), 7.07 (1H, t, C_6H_3CO), 7.62 (1H, d, C_6H_3CO), 7.75 (1H, d, C_6H_3CO).

5.31. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(4,5-dihydro-1*H*-imidazol-2-ylamino)piperidin-1-yl}benzoylamino]propionate (17a)

The carboxylic acid **16a** (28.0 mg, 0.100 mmol), and 1,1′-carbonyldiimidazole (CDI) (16.0 mg, 0.100 mmol) were dissolved in DMF (2.0 mL) and stirred for 1.5 h at room temperature. The mixture was added hydrochloride salt

of *t*-butyl (2*S*)-*N*-benzenesulfonyl-2,3-diaminopropionate **6a** (60.0 mg, 0.200 mmol) and warmed up to 60 °C. After 15 h, the solvent was removed in vacuo and the residue was purified by silica gel preparative TLC ($CHCl_3$/MeOH = 8:2) to give **17a** (16.6 mg, 0.0291 mmol, 29%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.23 (9H, s, *t*-Bu), 1.63 (2H, m, piperidine), 2.02 (2H, br d, piperidine), 2.96 (2H, br t, piperidine), 3.48 (1H, dd, $CONHCH_2CH$), 3.53 (1H, m, piperidine), 3.64 (1H, dd, $CONHCH_2CH$), 3.73 (4H, s, CH_2CH_2), 3.89 (2H, br d, piperidine), 4.11 (1H, dd, $CONHCH_2CH$), 6.98 (2H, d, C_6H_4CO), 7.47 (2H, t, C_6H_5), 7.54 (1H, t, C_6H_5), 7.67 (2H, d, C_6H_4CO), 7.83 (2H, br d, C_6H_5); $[\alpha]_D^{27}$ +20° (*c* 0.40, MeOH).

5.32. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[3-fluoro-4-{4-(4,5-dihydro-1*H*-imidazol-2-ylamino)piperidin-1-yl}-benzoylamino]propionate (17b)

The title compound (8.20 mg, 0.0139 mmol, 4%) was synthesized from **16b** (100 mg, 0.312 mmol) following the general procedure for **17a**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.23 (9H, s, *t*-Bu), 1.72 (2H, m, piperidine), 2.06 (2H, br d, piperidine), 2.89 (2H, br t, piperidine), 3.47 (1H, dd, $CONHCH_2CH$), 3.49 (1H, m, piperidine), 3.57 (2H, br d, piperidine), 3.66 (1H, dd, $CONHCH_2CH$), 3.73 (4H, s, CH_2CH_2), 4.12 (1H, dd, $CONHCH_2CH$), 7.06 (1H, t, C_6H_3CO), 7.48 (2H, m, C_6H_5), 7.54 (2H, m, C_6H_5 and C_6H_3CO), 7.67 (1H, ddt, C_6H_3CO), 7.83 (2H, br d, C_6H_5); TSPMS *m*/*z* 589 $(M+H)^+$.

5.33. (2*S*)-Benzenesulfonylamino-3-[4-{4-(4,5-dihydro-1*H*-imidazol-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (13)

The compound **17a** (28.0 mg, 0.0490 mmol) afforded **13** (11.0 mg, 0.0214 mmol, 44%) as a colorless solid following the general procedure for **1** (Step 1): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (2H, br q, piperidine), 1.85 (2H, m, piperidine), 2.72 (2H, m, piperidine), 3.08 (1H, br t, $CONHCH_2CH$), 3.35 (2H, m, $CONHCH_2CH$ and piperidine), 3.56 (4H, s, CH_2CH_2), 3.64 (3H, m, piperidine and $CONHCH_2CH$), 6.89 (2H, d, C_6H_4CO), 7.54 (2H, br t, C_6H_5), 7.60 (3H, m, C_6H_5 and C_6H_4CO), 7.81 (2H, br d, C_6H_5); TSPMS *m*/*z* 515 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{24}H_{30}N_6O_5S$: 515.2077, found: 515.2070; $[\alpha]_D^{23}$ +31° (*c* 0.16, DMSO).

5.34. (2*S*)-Benzenesulfonylamino-3-[3-fluoro-4-{4-(4,5-dihydro-1*H*-imidazol-2-ylamino)piperidin-1-yl}benzoylamino]-propionic acid (14)

The title compound (7.40 mg, 0.0139 mmol, 48%) was synthesized from **17b** following the general procedure for **1** (Step 1): ^{1}H NMR (400 MHz, CD_3OD) δ 1.74 (2H, dd, piperidine), 2.06 (2H, br d, piperidine), 2.89 (2H, br t, piperidine), 3.47 (1H, m, piperidine), 3.49 (1H, dd, $CONHCH_2CH$), 3.57 (2H, br d, piperidine), 3.70 (1H, dd, $CONHCH_2CH$), 3.73 (4H, s, CH_2CH_2), 4.07 (1H, dd, $CONHCH_2CH$), 7.05 (1H, t, C_6H_3CO), 7.49 (5H, m, C_6H_5 and C_6H_3CO), 7.83 (2H, br d, C_6H_5); TSPMS *m*/*z* 533 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{24}H_{29}FN_6O_5S$: 533.1982, found: 533.1988; $[\alpha]_D^{23}$ +37° (*c* 0.06, MeOH).

5.35. 4-[4-[3-{2-(*t*-Butoxycarbonylamino)butyl}thioureido]piperidin-1-yl]-3- fluorobenzoate (19a)

The title compound was synthesized from **4a** and *N*-Boc-4-isothiocyanatobutylamine following the general procedure for **15a**: FABMS *m*/*z* 479 $(M+H)^+$.

5.36. 4-{4-(4,5,6,7-Tetrahydro-1*H*-[1,3]diazepin-2-ylamino)piperidin-1-yl}benzoic acid (20a)

The title compound (4.50 mg, 0.0129 mmol, 35% from **4a**) was synthesized from **19a** following the general procedure for **16a**: FABMS *m*/*z* 317 $(M+H)^+$.

5.37. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(4,5,6,7-tetrahydro-1*H*-[1,3] diazepin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (21a)

The title compound (2.00 mg, 0.00334 mmol, 26%) was synthesized from **20a** (4.5 mg, 0.0129 mmol) following the general procedure for **17a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (9H, s, *t*-Bu), 1.55 (2H, m, piperidine), 1.61 (4H, m, $CH_2CH_2CH_2CH_2$), 1.94 (2H, br d, piperidine), 2.85 (2H, br t, piperidine), 3.22 (4H, m, $CH_2CH_2CH_2CH_2$), 3.52 (2H, m, piperidine), 3.70 (1H, m, $CONHCH_2CH$), 3.78 (1H, m, $CONHCH_2CH$), 3.88 (1H, m, piperidine), 4.09 (1H, dd, $CONHCH_2CH$), 6.59 (2H, d, C_6H_4CO), 7.41 (2H, br t, C_6H_5), 7.49 (1H, br t, C_6H_5), 7.66 (2H, d, C_6H_4CO), 7.85 (2H, br d, C_6H_5); TSPMS *m*/*z* 599 $(M+H)^+$.

5.38. (2*S*)-Benzenesulfonylamino-3-[4-{4-(4,5,6,7-tetrahydro-1*H*-[1,3]diazepin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (18)

The title compound (1.70 mg, 0.00314 mmol, 94%) was synthesized from **21a** following the general procedure for **1** (Step 1): ^{1}H NMR (400 MHz, CD_3OD) δ 1.61 (2H, br q, piperidine), 1.70 (4H, m, $CH_2CH_2CH_2CH_2$), 1.98 (2H, br d, piperidine), 2.94 (2H, ddd, piperidine), 3.28 (4H, m, $CH_2CH_2CH_2CH_2$), 3.60 (3H, m, $CONHCH_2CH$ and piperidine), 3.73 (1H, dd, $CONHCH_2CH$), 3.86 (2H, br d, piperidine), 6.96 (2H, d, C_6H_4CO), 7.48 (2H, br t, C_6H_5), 7.54 (1H, br t, C_6H_5), 7.71 (2H, d, C_6H_4CO), 7.86 (2H, br d, C_6H_5); FABMS *m*/*z* 543 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{26}H_{34}N_6O_5S$: 543.2390, found: 543.2401; $[\alpha]_D^{25}$ +53° (*c* 0.24, MeOH/concd NH_4OH = 10:1).

5.39. 4-(1*H*-Benzimidazol-2-ylamino)piperidine (24)

2-Chlorobenzimidazole (5.00 g, 32.8 mmol) and **23** (11.2 mL, 65.5 mmol) were combined and heated at 170 °C for 5 h. The mixture was cooled to room temperature and added heated $CHCl_3$ (200 mL). The resulting solution was added aqueous $NaHCO_3$ (500 mL). After extraction twice, the combined organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was added AcOEt (100 mL) and the resulting precipitate was filtered by glassfilter. The solids were rinsed with AcOEt (100 mL) and dried to give the coupled compound (6.80 g, 23.5 mmol, 72%) as a faint brown solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.27 (3H, t, CH_2CH_3), 1.47 (2H, ddd, piperidine), 2.07 (2H,

br d, piperidine), 3.03 (2H, m, piperidine), 3.79 (1H, tt, piperidine), 4.13 (4H, m, piperidine and CH_2CH_3), 7.03 (2H, dd, benzimidazole), 7.23 (2H, dd, benzimidazole); TSPMS *m*/*z* 289 $(M+H)^+$.

This compound (150 mg, 0.520 mmol) was dissolved in 47% HBr (2.5 mL) and heated at reflux. After 7.5 h, the solvent was removed in vacuo. The residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) to give **24** (91.0 mg, 0.421 mmol, 81%) as a colorless solid: ^1H NMR (400 MHz, CD_3OD) δ 1.47 (2H, dq, piperidine), 2.07 (2H, m, piperidine), 2.74 (2H, dt, piperidine), 3.10 (2H, dt, piperidine), 3.72 (1H, tt, piperidine), 6.95 (2H, dd, benzimidazole), 7.17 (2H, dd, benzimidazole); EIMS *m*/*z* 216 $(M)^+$.

5.40. 4-{4-(1*H*-Benzimidazol-2-ylamino)piperidin-1-yl}benzoic acid (25)

A mixture of **2a** (0.770 mL, 5.23 mmol), **24** (1.13 g, 5.23 mmol), and DMSO (5.2 mL) was heated to 140 °C and stirred for 6.5 h, then cooled. The residue was partitioned between CH_2Cl_2 and brine (300 mL). The aqueous phase was further extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The resulting residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 25:1) to provide ethyl 4-{4-(1*H*-benzimidazol-2-ylamino)piperidin-1-yl}benzoate (941 mg, 2.58 mmol, 49%) as a colorless solid: ^1H NMR (400 MHz, CD_3OD) δ 1.36 (3H, t, CH_2CH_3), 1.63 (2H, m, piperidine), 2.13 (2H, br d, piperidine), 3.07 (2H, br t, piperidine), 3.84 (1H, tt, piperidine), 3.92 (2H, br d, piperidine), 4.30 (2H, q, CH_2CH_3), 6.97 (2H, br d, C_6H_4), 7.00 (2H, dd, benzimidazole), 7.21 (2H, dd, benzimidazole), 7.87 (2H, br d, C_6H_4); TSPMS *m*/*z* 365 $(M+H)^+$.

To a solution of this benzoate (103 mg, 0.283 mmol) in a mixture of THF (9.0 mL) and MeOH (3.0 mL) 1 M NaOH (3.0 mL) was added. The reaction mixture was stirred for 3.5 h at 40 °C and concentrated. The residue was purified using silica gel flash column chromatography ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) to give **25** (92.3 mg, 0.274 mmol, 97%) as a faint yellow solid: ^1H NMR (400 MHz, DMSO-d_6) δ 1.53 (2H, br q, piperidine), 2.02 (2H, br d, piperidine), 3.00 (2H, br t, piperidine), 3.83 (1H, m, piperidine), 3.89 (2H, br d, piperidine), 6.84 (2H, m, benzimidazole), 6.97 (2H, d, C_6H_4), 7.11 (2H, m, benzimidazole), 7.75 (2H, d, C_6H_4); TSPMS *m*/*z* 337 $(M+H)^+$.

5.41. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(1*H*-benzimidazol-2-ylamino)piperidin-1-yl}benzoylamino]-propionionate (26)

The title compound (62.2 mg, 0.101 mmol, 67%) was synthesized from **25** (50.0 mg, 0.149 mmol) following the general procedure for **7b**: ^1H NMR (400 MHz, $CDCl_3$) δ 1.23 (9H, s, *t*-Bu), 1.26 (2H, m, piperidine), 1.93 (2H, br d, piperidine), 2.72 (2H, t, piperidine), 3.46 (2H, br d, piperidine), 3.64 (1H, m, piperidine), 3.81 (2H, m, $CONHCH_2CH$), 4.07 (1H, dd, $CONHCH_2CH$), 6.59 (2H, d, C_6H_4), 7.00 (2H, dd, benzimidazole), 7.17 (1H, m, benzimidazole), 7.28 (1H, m, benzimidazole), 7.37 (2H, t, C_6H_5), 7.48 (1H, t, C_6H_5), 7.58 (2H, d, C_6H_4), 7.77 (2H, br d, C_6H_5); TSPMS *m*/*z* 619 $(M+H)^+$; $[\alpha]_D^{24}$ +27° (*c* 0.97, MeOH).

5.42. (2*S*)-Benzenesulfonylamino-3-[4-{4-(1*H*-benzimidazol-2-ylamino)piperidin-1-yl}benzoylamino]-propionic acid (22)

The title compound (36.3 mg, 0.0645 mmol, 67%) was synthesized from the amide **26** (60.0 mg, 0.0970 mmol) following the general procedure for **1** (Step 1): ^1H NMR (400 MHz, CD_3OD) δ 1.54 (2H, br q, piperidine), 2.01 (2H, br d, piperidine), 2.95 (2H, br t, piperidine), 3.34 (2H, m, $CONHCH_2CH$), 3.85 (2H, m, piperidine and $CONHCH_2CH$), 3.86 (2H, br d, piperidine), 6.89 (2H, m, benzimidazole), 6.96 (2H, d, C_6H_4), 7.14 (2H, dd, benzimidazole), 7.47 (2H, m, C_6H_5), 7.54 (1H, br t, C_6H_5), 7.62 (2H, d, C_6H_4), 7.76 (2H, br d, C_6H_5); TSPMS *m*/*z* 563 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{28}H_{30}N_6O_5S$: 563.2077, found: 563.2072; $[\alpha]_D^{26}$ +80° (*c* 1.1, MeOH/concd NH_4OH = 9:1).

5.43. 4-{(1*H*-Imidazo[4,5-*b*]pyridin-2-yl)amino}piperidine (28)

The title compound (226 mg, 1.04 mmol, 24% in two steps) was synthesized from 2-chloro-1*H*-imidazo[4,5-*b*]pyridine[9] following the general procedure for **24**, except that **28** was purified by Amberlyst 15 (MeOH/concd NH_4OH/water = 4:3:1): ^1H NMR (400 MHz, CD_3OD) δ 1.50 (2H, dq, piperidine), 2.07 (2H, br d, piperidine), 2.74 (2H, dt, piperidine), 3.10 (2H, dt, piperidine), 3.80 (1H, tt, piperidine), 6.96 (1H, dd, C_6H_3N), 7.46 (1H, dd, C_6H_3N), 7.92 (1H, dd, C_6H_3N); TSPMS *m*/*z* 218 $(M+H)^+$.

5.44. 4-[4-{(1*H*-Imidazo[4,5-*b*]pyridin-2-yl)amino}piperidin-1-yl]benzoic acid (29)

The title compound was synthesized from **28** following the general procedure for **25**: FABMS *m*/*z* 338 $(M+H)^+$.

5.45. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-[4-{(1*H*-imidazo[4,5-*b*]pyridin-2-yl)amino}piperidin-1-yl]benzoyl-amino]propionate (30)

The title compound (83.2 mg, 0.134 mmol, 23% from **28**) was synthesized from **29** following the general procedure for **7b**: ^1H NMR (400 MHz, DMSO-d_6) δ 1.12 (9H, s, *t*-Bu), 1.56 (2H, br q, piperidine), 2.01 (2H, br d, piperidine), 2.95 (2H, m, piperidine), 3.31 (1H, m, $CONHCH_2CH$), 3.44 (1H, m, $CONHCH_2CH$), 3.87 (3H, m, piperidine), 4.20 (1H, br t, $CONHCH_2CH$), 6.88 (1H, m, C_6H_3N), 6.96 (2H, d, C_6H_4CO), 7.36 (1H, m, C_6H_3N), 7.51 (2H, t, C_6H_5), 7.57 (1H, t, C_6H_5), 7.64 (2H, d, C_6H_4CO), 7.76 (2H, br d, C_6H_5), 8.17 (1H, t, C_6H_3N); TSPMS *m*/*z* 620 $(M+H)^+$; $[\alpha]_D^{24}$ +29° (*c* 0.85, MeOH).

5.46. (2*S*)-Benzenesulfonylamino-3-[4-[4-{(1*H*-imidazo[4,5-*b*]pyridin-2-yl)amino}piperidin-1-yl]benzoyl-amino]propionic acid (27)

The title compound (38.0 mg, 0.0675 mmol, 99%) was synthesized from **30** (42.0 mg, 0.0679 mmol) following

the general procedure for **1** (Step 1): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.57 (2H, br q, piperidine), 2.01 (2H, br d, piperidine), 2.96 (2H, t, piperidine), 3.33 (1H, m, CONHCH_2CH), 3.45 (1H, m, CONHCH_2CH), 3.89 (3H, m, piperidine), 3.96 (1H, t, CONHCH$_2$CH), 6.85 (1H, br t, C_6H_3N), 6.96 (2H, d, C_6H_4CO), 7.38 (1H, dd, C_6H_3N), 7.46 (2H, m, C_6H_5), 7.54 (1H, br t, C_6H_5), 7.62 (2H, d, C_6H_4CO), 7.75 (2H, m, C_6H_5), 8.18 (1H, br t, C_6H_3N); FABMS *m*/*z* 564 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{29}H_{29}N_7O_5S$: 564.2029, found: 564.2022; $[\alpha]_D^{23}$ +53° (*c* 0.35, MeOH/concd NH_4OH = 10:1).

5.47. 4-[4-{(*t*-Butoxycarbonyl)amino}piperidin-1-yl]benzoic acid (32)

A mixture of **4a** (154 mg, 0.621 mmol), 1,4-dioxane (2.0 mL), H_2O (1.0 mL), 1 M NaOH (1.0 mL), and Boc_2O (170 mg, 0.778 mmol) was stirred for 0.5 hr and concentrated. To this THF (9.0 mL), MeOH (3.0 mL), and 1 M NaOH (6.0 mL) were added. The mixture was stirred for 4.5 h at 50 °C, then cooled to room temperature and acidified to pH 6 using 1 M aqueous HCl. The mixture was added H_2O (100 mL) and extracted with AcOEt three times. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated to afford **32** (168 mg, 0.527 mmol, 85% in two steps) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.44 (9H, s, *t*-Bu), 1.51 (2H, m, piperidine), 1.93 (2H, br d, piperidine), 2.95 (2H, dt, piperidine), 3.55 (1H, m, piperidine), 3.88 (2H, br d, piperidine), 6.94 (2H, d, C_6H_4), 7.85 (2H, d, C_6H_4); EIMS *m*/*z* 320 $(M)^+$.

5.48. *t*-Butyl 3-{4-(4-aminopiperidin-1-yl)benzoylamino}-(2*S*)-(benzenesulfonylamino)propionate (33)

The carboxylic acid **32** (81.0 mg, 0.254 mmol) afforded *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-[4-{(*t*-butoxycarbonyl)amino}piperidin-1-yl]benzoylamino]propionate (111 mg, 0.184 mmol, 73%) following the procedure for **7b**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.28 (9H, s, *t*-Bu), 1.45 (9H, s, *t*-Bu), 1.48 (2H, br d, piperidine), 2.05 (2H, br d, piperidine), 2.95 (2H, br t, piperidine), 3.55 (1H, ddd, CONHCH_2CH), 3.68 (1H, m, piperidine), 3.77 (2H, br d, piperidine), 3.89 (2H, m, CONHCH_2CH), 6.89 (2H, d, C_6H_4), 7.50 (2H, m, C_6H_5), 7.57 (1H, m, C_6H_5), 7.69 (2H, d, C_6H_4), 7.84 (2H, m, C_6H_5); TSPMS *m*/*z* 603 $(M+H)^+$.

The amide (10.0 mg, 0.0166 mmol) and saturated HCl/MeOH were combined and stirred at room temperature for 4 h. The mixture was poured into a mixture of MeOH (5.0 mL) and concd NH_4OH (5.0 mL). The solvent was evaporated in vacuo and the residue was purified by silica gel preparative TLC ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) to give **33** (7.00 mg, 0.0139 mmol, 84%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.22 (9H, s, *t*-Bu), 1.47 (2H, dq, piperidine), 1.93 (2H, br d, piperidine), 2.87 (3H, m, piperidine), 3.49 (1H, dd, CONHCH_2CH), 3.64 (1H, dd, CONHCH_2CH), 3.88 (2H, br d, piperidine), 4.11 (1H, dd, CONHCH$_2$CH), 6.95 (2H, br d, C_6H_4CO), 7.47 (2H, br t, C_6H_5), 7.54 (1H, br t, C_6H_5), 7.65 (2H, br d, C_6H_4CO), 7.83 (2H, m, C_6H_5); TSPMS *m*/*z* 502 $(M+H)^+$.

5.49. (2*S*)-Benzenesulfonylamino-3-{4-(4-guanigino-piperidin-1-yl)benzoylamino}propionic acid (31)

To a suspension of the amine **33** (18.8 mg, 0.0374 mmol) in a mixture of 1,4-dioxane (0.080 mL) and water (0.080 mL) *i*-Pr_2NEt (27 μL, 0.150 mmol) and 1*H*-pyrazole-1-carboxamidine nitrate (22.0 mg, 0.150 mmol) were added. The mixture was stirred for 31 h at room temperature. After removing the solvent, the residue was purified by silica gel preparative TLC ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) to give the guanidine (17.1 mg, 0.0314 mmol, 84%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.23 (9H, s, *t*-Bu), 1.62 (2H, dq, piperidine), 2.04 (2H, br d, piperidine), 2.99 (2H, ddd, piperidine), 3.48 (1H, dd, CONHCH_2CH), 3.63 (2H, m, CONHCH_2CH and piperidine), 3.88 (2H, br d, piperidine), 4.12 (1H, dd, CONHCH$_2$CH), 6.98 (2H, br d, C_6H_4CO), 7.47 (2H, br t, C_6H_5), 7.54 (1H, br t, C_6H_5), 7.68 (2H, d, C_6H_4CO), 7.83 (2H, m, C_6H_5); FABMS *m*/*z* 544 $(M+H)^+$; $[\alpha]_D^{25}$ +24° (*c* 0.85, MeOH).

The ester (17.0 mg, 0.0312 mmol) afforded **31** (10.2 mg, 0.0209 mmol, 67%) following the general procedure for **1** (Step 1): ^{1}H NMR (400 MHz, CD_3OD) δ 1.61 (2H, br q, piperidine), 2.01 (2H, br d, piperidine), 2.96 (2H, t, piperidine), 3.60 (3H, m, CONHCH_2CH and piperidine), 3.74 (1H, dd, CONHCH$_2$CH), 3.85 (2H, br d, piperidine), 6.96 (2H, br d, C_6H_4CO), 7.47 (2H, br t, C_6H_5), 7.54 (1H, br t, C_6H_5), 7.70 (2H, br d, C_6H_4CO), 7.85 (2H, dd, C_6H_5); TSPMS *m*/*z* 489 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{22}H_{28}N_6O_5S$: 489.1920, found: 489.1924; $[\alpha]_D^{23}$ +126° (*c* 0.05, DMSO).

5.50. *t*-Butyl 3-amino-(2*S*)-hydroxypropionate (6d)

In a Fisher–Porter tube, to a mixture of L-isoserine (4.00 g, 38.1 mmol) and DME (76 mL) concd H_2SO_4 (3.2 mL) was added. The mixture was cooled to −78 °C and added isobutylene (38 mL). The tube was sealed and the cooling bath was removed. After 48 h, isobutylene was removed at room temperature. The mixture was poured into ice/water (128 mL) and washed with Et_2O twice. The aqueous phase was alkalized to pH 7 using 6 M aqueous NaOH at 0 °C, saturated with NaCl, and extracted with $CHCl_3$ (100 mL) four times. The combined extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH/concd NH_4OH = 400:20:1 to 100:10:1) to prepare **6d** (191 mg, 1.19 mmol, 3%) as a colorless crystal: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (9H, s, *t*-Bu), 2.91 (1H, dd, CH_2NH_2), 3.02 (1H, dd, CH_2NH_2), 4.05 (1H, dd, COCH); FABMS *m*/*z* 162 $(M+H)^+$.

5.51. *t*-Butyl 4-amino-(2*S*)-*t*-butoxybutylate (6e)

The title compound (161 mg, 0.697 mmol, 2%) was synthesized from 4-amino-(2*S*)-hydroxybutylic acid[11] (4.00 g, 33.6 mmol) following the general procedure **6d**; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (9H, s, *t*-Bu), 1.46 (9H, s, CO_2-*t*-Bu), 1.75 (2H, q, $CH_2CH_2NH_2$), 2.76 (1H, ddd, CH_2NH_2), 2.83 (1H, ddd, CH_2NH_2), 3.95 (1H, dd, COCH); TSPMS *m*/*z* 232 $(M+H)^+$.

5.52. (2*R*)-Benzenesulfonylamino-3-[3-fluoro-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (34)

The title compound (53.4 mg, 0.0978 mmol, 58% in three steps) was synthesized from **5b** and **6b** following the general procedure for **8**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.68 (2H, br q, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 2.02 (2H, br d, piperidine), 2.87 (2H, br t, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.48 (1H, m, piperidine), 3.52 (2H, br d, piperidine), 3.57 (1H, dd, $CONHCH_2CH$), 3.65 (1H, dd, $CONHCH_2CH$), 3.73 (1H, dd, $CONHCH_2CH$), 7.04 (1H, t, C_6H_3CO), 7.53 (5H, m, C_6H_3CO and C_6H_5), 7.86 (2H, br d, C_6H_5); TSPMS *m*/*z* 547 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}FN_6O_5S$: 547.2139, found: 547.2148; $[\alpha]_D^{22}$ −100° (*c* 0.32, MeOH/CH_2Cl_2 = 1:1).

5.53. 3-[4-{4-(1*H*-Benzimidazol-2-ylamino)piperidin-1-yl}-benzoylamino]-(2*S*)-{(benzyloxycarbonyl)amino}propionic acid (35)

The title compound (7.00 mg, 0.0124 mmol, 20%) was synthesized from **25** and **6c** following the general procedure for **1** (Step 1): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54 (2H, br q, piperidine), 2.01 (2H, br d, piperidine), 2.96 (2H, br t, piperidine), 3.56 (2H, m, $CONHCH_2CH$), 3.83 (3H, m, piperidine and $CONHCH_2CH$), 4.18 (1H, m, piperidine), 5.02 (2H, s, $CH_2C_6H_5$), 6.97 (2H, d, C_6H_4CO), 7.11 (4H, m, benzimidazole and $CH_2C_6H_5$), 7.32 (5H, m, benzimidazole and $CH_2C_6H_5$), 7.38 (2H, d, C_6H_4CO); FABMS *m*/*z* 557 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{30}H_{32}N_6O_5$: 557.2512, found: 557.2504; $[\alpha]_D^{23}$ −9.5° (*c* 0.35, MeOH/concd NH_4OH = 10:1).

5.54. (2*S*)-Hydroxy-3-[4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (36)

The title compound was synthesized from **5a** and **6d** following the general procedure **1**: ^{1}H NMR (400 MHz, D_2O) (as hydrochloride) δ 1.65 (2H, m, piperidine), 1.77 (2H, quintet, tetrahydropyrimidine), 2.03 (2H, br d, piperidine), 3.17 (4H, t, tetrahydropyrimidine), 3.18 (2H, br t, piperidine), 3.51 (1H, m, piperidine), 3.57 (2H, dd, $CONHCH_2$), 3.61 (2H, br d, piperidine), 4.26 (1H, t, $CONHCH_2CH$), 7.25 (2H, d, C_6H_4), 7.66 (2H, d, C_6H_4); FABMS *m*/*z* 390 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{19}H_{27}N_5O_4$: 390.2141, found: 390.2143; $[\alpha]_D^{25}$ −3° (*c* 1.2, H_2O).

5.55. (2*S*)-Acetoxy-3-[4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (39)

The compound **39** was generated as a by-product in the course of the final synthetic step of **36**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.57 (2H, br q, piperidine), 1.95 (2H, quintet, tetrahydropyrimidine), 1.96 (2H, br d, piperidine), 2.09 (3H, s, Ac), 2.90 (2H, br t, piperidine), 3.36 (4H, t, tetrahydropyrimidine), 3.49 (1H, m, piperidine), 3.74 (1H, dd, $CONHCH_2$), 3.78 (2H, br d, piperidine), 3.80 (1H, dd, $CONHCH_2$), 5.06 (1H, dd, $CONHCH_2CH$), 6.92 (2H, d, C_6H_4), 7.69 (2H, d, C_6H_4); TSPMS *m*/*z* 432 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{21}H_{29}N_5O_5$: 432.2247, found: 432.2245; $[\alpha]_D^{25}$ −23° (*c* 0.10, MeOH).

5.56. (2*S*)-Hydroxy-4-[4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]butylic acid (37)

The title compound was synthesized from **5a** and **6e** following the general procedure for **1**: ^{1}H NMR (400 MHz, CD_3OD–20% ND_4OD/D_2O = 5:1) δ 1.60 (2H, m, piperidine), 1.86 (1H, ddd, $CONHCH_2CH_2$), 1.96 (2H, quintet, tetrahydropyrimidine), 2.03 (2H, br d, piperidine), 2.07 (1H, m, $CONHCH_2CH_2$), 2.98 (2H, br t, piperidine), 3.38 (4H, t, tetrahydropyrimidine), 3.44–3.62 (3H, m, piperidine, $CONHCH_2$), 3.83 (2H, br d, piperidine), 4.00 (1H, dd, $CONHCH_2CH_2CH$), 7.00 (2H, d, C_6H_4), 7.73 (2H, d, C_6H_4); TSPMS *m*/*z* 404 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{20}H_{29}N_5O_4$: 404.2298, found: 404.2290; $[\alpha]_D^{25}$ −7° (*c* 1.0, MeOH/H_2O/concd NH_4OH = 8:1:1).

5.57. *t*-Butyl (2*S*)-benzenesulfonylamino-3-{*N*-(2-chloroethyl)-2,2,2-trifluoroacetamido}propionate (40)

The compound **6a** (400 mg, 1.33 mmol) was dissolved in MeOH (20 mL). 2-Chloroacetaldehyde (105 mg, 1.33 mmol) was added, and then $CHCl_3$ (20 mL) and a drop of AcOH were added. $NaBCNH_3$ (251 mg, 3.99 mmol) was added, and the reaction solution was kept at room temperature for 4 h. The solution was evaporated to afford a residue. Saturated $NaHCO_3$ (40 mL) was added, and a desired compound was extracted with $CHCl_3$ (40 mL) twice. The organic layer was concentrated to afford crude 2-chloroethyl derivative of **6a**.

To the above crude compound, $NaHCO_3$ (469 mg) and 1,4-dioxane (17.5 mL) were added. Trifluoromethanesulfonic anhydride (588 mg, 2.08 mmol) was added, and the mixture was gently stirred at room temperature for 16 h. Concd NH_4OH (0.20 mL) was added and the mixture was evaporated. Saturated $NaHCO_3$ (150 mL) was added, and the crude compound was extracted with $CHCl_3$ (150 mL) twice. The organic layer was dried and concentrated. The residue was purified by silica gel flash column chromatography (toluene/AcOEt = 5:1) to prepare **40** (379 mg, 0.944 mmol, 71% in two steps) as an amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (9H, s, *t*-Bu) 3.80 (7H, m, CH_2CH_2 and CH_2CH), 7.51 (2H, dd, C_6H_5), 7.59 (1H, t, C_6H_5), 7.83 (2H, br d, C_6H_5); FABMS *m*/*z* 459 $(M+H)^+$.

5.58. *t*-Butyl 1-benzenesulfonylpiperazine-(2*S*)-carboxylate (6f)

DMF (5.9 mL) was added to **40** (294 mg, 0.641 mmol). DBU (97.5 mg, 0.641 mmol) was added and the solution was kept at room temperature for 1 h. The mixture was extracted with AcOEt (100 mL). The organic layer was washed with aqueous 5% $KHSO_4$, saturated $NaHCO_3$, and brine. Dried organic layer was evaporated. The residue

was purified by silica gel flash column chromatography (toluene/AcOEt = 5:1) to prepare trifluoroacetoamido of **6f** (234 mg, 0.554 mmol, 91%) as an amorphous.

The above compound (50.0 mg, 0.118 mmol) was dissolved in 1,4-dioxane (4.0 mL) with concd NH_4OH (2.0 mL). The solution was kept at room temperature for 16 h. Evaporated solution was purified by silica gel preparative TLC ($CHCl_3$/MeOH = 19:1) to give **6f** (35.0 mg, 0.107 mmol, 91%) as a colorless syrup: ^{1}H NMR (400 MHz, CD_3OD) δ 1.37 (9H, s, *t*-Bu), 2.59 (1H, dt, piperidine), 2.84 (1H, dd, piperidine), 2.90 (1H, br d, piperidine), 3.34 (2H, m, piperidine), q3.56 (1H, dt, piperidine), 4.39 (1H, br d, piperidine), 7.53 (2H, br t, C_6H_5), 7.62 (1H, br t, C_6H_5), 7.81 (2H, m, C_6H_5); TSPMS *m*/*z* 327 $(M+H)^+$.

5.59. 1-Benzenesulfonyl-4-[4-{4-(1*H*-benzimidazol-2-yl-amino)piperidin-1-yl}benzoyl]piperazine-(2*S*)-carboxylic acid (38)

The title compound was synthesized from **25** and **6f** following the general procedure **22**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.72 (2H, m, piperidine), 2.09 (2H, br d, piperidine), 2.91 (2H, br t, piperidine), 3.33 (4H, m, piperidine and piperazine), 3.53 (1H, m, piperidine), 3.72 (2H, m, piperazine), 3.84 (2H, m, piperazine), 4.49 (1H, m, piperazine), 6.92 (2H, br d, C_6H_4), 7.22 (5H, m, C_6H_5 and benzimidazole), 7.42 (2H, m, benzimidazole), 7.52 (2H, m, C_6H_5), 7.90 (2H, br d, C_6H_4); FABMS *m*/*z* 589 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{30}H_{32}N_6O_5S$: 589.2233, found: 589.2224; $[\alpha]_D^{27}$ −28° (*c* 0.45, MeOH/$CHCl_3$ = 1:1).

5.60. *t*-Butyl (2*S*)-amino-3-[4-[4-{(1-*t*-butoxycarbonyl-1*H*-benzimidazol-2-yl)amino}piperidin-1-yl]benzoyl-amino]propionate (41)

The carboxylic acid **25** (42.0 mg, 0.125 mmol) and **6c** afforded the desired amide (58.8 mg, 0.0950 mmol, 77%) as a colorless solid following the general procedure for **7b**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.42 (9H, s, *t*-Bu), 1.67 (2H, br q, piperidine), 2.15 (2H, m, piperidine), 3.06 (2H, br t, piperidine), 3.72 (2H, d, CONHC*H*$_2$CH), 3.85 (3H, m, piperidine), 4.36 (1H, t, CONHCH$_2$C*H*), 5.08 (2H, dd, C*H*$_2$C$_6$H$_5$), 6.99 (2H, d, C_6H_4CO), 7.02 (2H, m, benzimidazole), 7.23 (2H, m, benzimidazole), 7.31 (5H, m, $CH_2C_6H_5$), 7.70 (2H, d, C_6H_4CO); TSPMS *m*/*z* 613 $(M+H)^+$; $[\alpha]_D^{23}$ −11° (*c* 1.0, MeOH).

The amide (50.0 mg, 0.0816 mmol) dissolved in CH_2Cl_2 (1.0 mL) were added Et_3N (34 μL, 0.245 mmol) and Boc_2O (47 μL, 0.204 mmol) at room temperature. After 1 h, the mixture was added aqueous $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2 three times. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel preparative TLC (CH_2Cl_2/MeOH = 7:1) to give *t*-butyl (2*S*)-benzyloxycarbonylamino-3-[4-[4-{(1-*t*-butoxycarbonyl-1*H*-benzimidazol-2-yl)amino}piperidin-1-yl]benzoylamino]propionate (60.0 mg, 0.0816 mmol, 100%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (9H, s, *t*-Bu), 1.70 (9H, s, *t*-Bu), 1.96 (2H, br s, piperidine), 2.27 (2H, br dd, piperidine), 3.12 (2H, br t, piperidine), 3.80 (4H, m, CONHC*H*$_2$CH and piperidine), 4.19 (1H, m, piperidine), 4.44 (1H, dd, CONHCH$_2$C*H*), 5.11 (2H, s, C*H*$_2$C$_6$H$_5$), 6.89 (2H, d, C_6H_4CO), 7.03 (1H, dt, benzimidazole), 7.19 (1H, dt, benzimidazole), 7.33 (5H, m, $CH_2C_6H_5$), 7.39 (1H, d, benzimidazole), 7.59 (1H, d, benzimidazole), 7.67 (2H, d, C_6H_4CO); FABMS *m*/*z* 713 $(M+H)^+$.

To a solution of the above compound (50.0 mg, 0.0701 mmol), THF (17.5 mL), water (5.0 mL), and AcOH (2.5 mL), 10% Pd/C (50 mg) was added. The mixture was hydrogenated under H_2 for 14 h at room temperature. The mixture was filtered through Celite, and solids were washed with EtOH. The filtrate was neutralized using 1 M aqueous NaOH, and the THF and EtOH were removed in vacuo at 30 °C. The resulting aqueous solution was saturated by NaCl and extracted with CH_2Cl_2 three times. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel preparative TLC (CH_2Cl_2/MeOH = 7:1) to give **41** (26.1 mg, 0.0451 mmol, 64%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (9H, s, *t*-Bu), 1.70 (9H, s, *t*-Bu), 1.72 (2H, br dq, piperidine), 2.28 (2H, br dd, piperidine), 3.12 (2H, ddd, piperidine), 3.47 (1H, m, CONHC*H*$_2$CH), 3.61 (1H, dd, CONHC*H*$_2$CH), 3.81 (3H, m, piperidine and CONHCH$_2$C*H*), 4.20 (1H, m, piperidine), 6.92 (2H, d, C_6H_4CO), 7.04 (1H, ddd, benzimidazole), 7.20 (1H, dt, benzimidazole), 7.39 (1H, d, benzimidazole), 7.60 (1H, d, benzimidazole), 7.70 (2H, d, C_6H_4CO); TSPMS *m*/*z* 579 $(M+H)^+$; $[\alpha]_D^{23}$ +14° (*c* 0.97, MeOH).

5.61. (2*S*)-(Butane-1-sulfonyl)amino-3-[4-{4-(1*H*-benzimidazol-2-ylamino)piperidin-1-yl}benzoylamino]-propionic acid (42)

Step 1: To a solution of the amine **41** (10.5 mg, 0.0181 mmol) in DMF (0.5 mL), *i*-Pr_2NEt (6.5 μl, 0.0362 mmol) and *n*-butanesulfonyl chloride (2.4 μL, 0.0181 mmol) were added. The mixture was stirred at room temperature for 1.5 h. Excess sulfonyl chloride was trapped by piperazine. After dilution with aqueous $NaHCO_3$ (30 mL), the mixture was extracted with AcOEt three times. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel preparative TLC (hexane/AcOEt = 1:2) to give *t*-butyl (2*S*)-(butane-1-sulfonyl)amino-3-[4-[4-{(1-*t*-butoxycarbonyl-1*H*-benzimidazol-2-yl)amino}piperidin-1-yl]benzoylamino]propionate (10.5 mg, 0.0150 mmol, 83%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, t, $CH_2CH_2CH_2CH_3$) 1.42 (2H, sextet, $CH_2CH_2CH_2CH_3$), 1.50 (9H, s, *t*-Bu), 1.70 (9H, s, *t*-Bu), 1.70 (2H, m, piperidine), 1.78 (2H, sextet, $CH_2CH_2CH_2CH_3$), 2.28 (2H, br dd, piperidine), 3.03 (2H, ddd, C*H*$_2$CH$_2$CH$_2$CH textsubscript3), 3.13 (2H, ddd, piperidine), 3.68 (1H, ddd, CONHC*H*$_2$CH), 3.79 (2H, br d, piperidine), 3.90 (1H, ddd, CONHC*H*$_2$CH), 4.19 (1H, dt, CONHCH$_2$C*H*), 4.21 (1H, m, piperidine), 6.92 (2H, d, C_6H_4CO), 7.13 (1H, dt, benzimidazole), 7.20 (1H, dt, benzimidazole), 7.39 (1H, d, benzimidazole), 7.59 (1H, d, benzimidazole), 7.70 (2H, d, C_6H_4CO); TSPMS *m*/*z* 699 $(M+H)^+$; $[\alpha]_D^{23}$ −1.5° (*c* 0.65, MeOH).

Step 2: The above compound (15.2 mg, 0.0217 mmol) afforded **42** (7.50 mg, 0.138 mmol, 64%) following the general procedure for **1** (Step 1): ^{1}H NMR (400 MHz, CD_3OD) δ 0.89 (3H, t, $CH_2CH_2CH_2CH_3$) 1.39 (2H, sextet, $CH_2CH_2CH_2CH_3$), 1.75 (4H, m, piperidine and $CH_2CH_2CH_2CH_3$), 2.12 (2H, br d, piperidine), 3.02 (4H, m, $CH_2CH_2CH_2CH_3$ and piperidine), 3.63 (1H, ddd, CONHCH_2CH), 3.75 (2H, m, piperidine and CONHCH_2CH), 3.88 (2H, br d, piperidine), 4.04 (1H, dd, $CONHCH_2CH$), 6.98 (2H, d, C_6H_4CO), 7.23 (2H, dd, benzimidazole), 7.36 (2H, dd, benzimidazole), 7.75 (2H, d, C_6H_4CO); TSPMS *m*/*z* 543 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{26}H_{34}N_6O_5S$: 543.2390, found: 543.2401; $[\alpha]_D^{23}$ +5.9° (*c* 0.38, MeOH/concd NH_4OH = 10:1).

5.62. 3-[4-{4-(1*H*-Benzimidazol-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-fluorobenzenesulfonyl)amino}propionic acid (43)

The title compound (10.0 mg, 0.0172 mmol, 38% in two steps) was synthesized from **41** and 4-fluorobenzenesulfonyl chloride following the general procedure for **42**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.72 (2H, br q, piperidine), 2.15 (2H, br d, piperidine), 3.04 (2H, br t, piperidine), 3.57 (1H, dd, CONHCH_2CH), 3.64 (1H, dd, CONHCH_2CH), 3.78 (1H, dd, $CONHCH_2CH$), 3.80 (1H, m, piperidine), 3.92 (2H, br d, piperidine), 6.99 (2H, d, C_6H_4CO), 7.17 (4H, m, benzimidazole and C_6H_4F), 7.29 (2H, dd, benzimidazole), 7.70 (2H, d, C_6H_4CO), 7.89 (2H, dd, C_6H_4F); FABMS *m*/*z* 581 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{28}H_{29}FN_6O_5S$: 581.1982, found: 581.1978; $[\alpha]_D^{27}$ +36° (*c* 0.50, MeOH/concd NH_4OH = 1:1).

5.63. 3-[4-{4-(1*H*-Benzimidazol-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(2,4,6-trimethylbenzenesulfonyl)amino}propionic acid (44)

The title compound (5.00 mg, 0.00828 mmol, 28% in two steps) was synthesized from **41** and 2,4,6-trimethylbenzenesulfonyl chloride following the general procedure for **42**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.70 (2H, br q, piperidine), 2.15 (2H, br d, piperidine), 2.22 (3H, s, C_6H_2Me), 2.63 (6H, s, C_6H_2Me), 3.05 (2H, br t, piperidine), 3.62 (3H, m, CONHCH_2CH), 3.81 (1H, m, piperidine), 3.92 (2H, br d, piperidine), 6.92 (2H, s, C_6H_2Me), 6.99 (2H, d, C_6H_4CO), 7.10 (2H, dd, benzimidazole), 7.27 (2H, dd, benzimidazole), 7.69 (2H, d, C_6H_4CO); TSPMS *m*/*z* 605 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{31}H_{36}N_6O_5S$: 605.2546, found: 605.2546; $[\alpha]_D^{27}$ +55° (*c* 0.25, MeOH/concd NH_4OH = 1:1).

5.64. 3-[4-{4-(1*H*-Benzimidazol-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-nitrobenzenesulfonyl)-amino}propionic acid (45)

The title compound (15.1 mg, 0.0248 mmol, 30% in two steps) was synthesized from **41** and 4-nitrobenzenesulfonyl chloride following the general procedure for **42**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.68 (2H, br q, piperidine), 2.18 (2H, br d, piperidine), 3.07 (2H, br t, piperidine), 3.58 (2H, m, CONHCH_2CH), 3.91 (4H, m, CONHCH_2CH and piperidine), 6.91 (2H, d, C_6H_4CO), 6.96 (2H, dd, benzimidazole), 7.20 (2H, dd, benzimidazole), 7.55 (2H, d, C_6H_4CO), 8.00 (2H, dt, $C_6H_4NO_2$), 8.14 (2H, dt, $C_6H_4NO_2$); TSPMS *m*/*z* 608 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{28}H_{29}N_7O_7S$: 608.1927, found: 608.1916; $[\alpha]_D^{28}$ +24° (*c* 0.20, MeOH/concd NH_4OH = 10:1).

5.65. (2*S*)-(4-Aminobenzenesulfonyl)amino-3-[4-{4-(1*H*-benzimidazol-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (46)

To a solution of **45** (15.0 mg, 0.0196 mmol) and EtOH (5.0 mL), 10% Pd/C (15 mg) was added. The mixture was hydrogenated under H_2 for 3 h at room temperature. The mixture was filtered through Celite, and solids were washed with MeOH. The filtrate was concentrated. The residue was purified by preparative TLC (CH_2Cl_2/MeOH = 7:1), and then LH-20 (MeOH/concd NH_4OH = 10:1) to give **46** (2.50 mg, 0.00433 mmol, 22%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.66 (2H, dq, piperidine), 2.16 (2H, br d, piperidine), 3.06 (2H, ddd, piperidine), 3.52 (1H, dd, CONHCH_2CH), 3.63 (1H, dd, CONHCH_2CH), 3.69 (1H, dd, $CONHCH_2CH$), 3.85 (1H, ddd, piperidine), 3.91 (2H, br d, piperidine), 6.62 (2H, br d, $C_6H_4NH_2$), 6.96 (2H, dd, benzimidazole), 7.01 (2H, d, C_6H_4CO), 7.19 (2H, dd, benzimidazole), 7.53 (2H, br d, $C_6H_4NH_2$), 7.73 (2H, d, C_6H_4CO); TSPMS *m*/*z* 578 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{28}H_{31}N_7O_5S$: 578.2186, found: 578.2184; $[\alpha]_D^{23}$ +101° (*c* 0.14, MeOH).

5.66. *t*-Butyl (2*S*)-amino-3-[3-fluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (47)

t-Butyl (2*S*)-(benzyloxycarbonyl)amino-3-[3-fluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-propionate was synthesized from **5b** and **6c** following the general procedure for **7b**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.43 (9H, s, *t*-Bu), 1.74 (2H, dq, piperidine), 2.11 (2H, br d, piperidine), 2.94 (2H, dt, piperidine), 3.58 (2H, br d, piperidine), 3.71 (2H, d, CONHCH_2CH), 3.95 (1H, tt, piperidine), 4.37 (1H, t, $CONHCH_2CH$), 5.08 (2H, dd, $CH_2C_6H_5$), 6.59 (1H, t, pyrimidine), 7.07 (1H, t, C_6H_3CO), 7.30 (5H, m, $CH_2C_6H_5$), 7.49 (1H, dd, C_6H_3CO), 7.55 (1H, dd, C_6H_3CO), 8.26 (2H, d, pyrimidine); TSPMS *m*/*z* 593 $(M+H)^+$.

To a solution of the above amide (500 mg, 0.844 mmol) and THF (10 mL), 10% Pd/C (100 mg) was added. The mixture was hydrogenated under H_2 for 12 h at room temperature. The mixture was filtered through Celite, and solids were washed with MeOH. The filtrate was concentrated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 7:1) to give **47** (334 mg, 0.729 mmol, 86%) as a colorless amorphous: ^{1}H NMR (400 MHz, CD_3OD) δ 1.43 (9H, s, *t*-Bu), 1.74 (2H, dq, piperidine), 2.11 (2H, br d, piperidine), 2.93 (2H, ddd, piperidine), 3.60 (5H, m, piperidine and CONHCH_2CH), 3.95 (1H, tt, piperidine), 6.59 (1H, t, pyrimidine), 7.09 (1H, t, C_6H_3CO), 7.54 (1H, dd, C_6H_3CO), 7.60 (1H, dd, C_6H_3CO), 8.26 (2H, d, pyrimidine); TSPMS *m*/*z* 459 $(M+H)^+$.

5.67. (2*S*)-Acetylamino-3-[3-fluoro-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (48)

The title compound was synthesized from **47** and acetyl chloride following the general procedure for **42** (Step 1) and then for **8**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.68 (2H, br q, piperidine), 1.96 (3H, s, Ac), 1.97 (2H, m, tetrahydropyrimidine), 2.02 (2H, br d, piperidine), 2.87 (2H, br t, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.47 (1H, m, piperidine), 3.53 (2H, br d, piperidine), 3.66 (1H, dd, CONHCH_2CH), 3.74 (1H, dd, CONHCH_2CH), 4.47 (1H, dd, CONHCH$_2$CH), 7.05 (1H, t, C_6H_3CO), 7.51 (1H, dd, C_6H_3CO), 7.56 (1H, dd, C_6H_3CO); FABMS *m*/*z* 449 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{21}H_{29}FN_6O_4$: 449.2313, found: 449.2309; $[\alpha]_D^{25}$ +9.3° (*c* 0.17, MeOH).

5.68. (2*S*)-(4-Aminobenzenesulfonyl)amino-3-[3-fluoro-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (49)

The title compound was synthesized from **47** and 4-nitrobenzenesulfonyl chloride following the general procedure for **42** (Step 1) and then for **8**: ^{1}H NMR (400 MHz, CD_3OD) (as hydrochloride) δ 1.67 (2H, br q, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 2.00 (2H, m, piperidine), 2.86 (2H, br t, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.48 (5H, m, piperidine and CONHCH_2CH), 3.64 (1H, dd, CONHCH_2CH), 3.69 (1H, m, CONHCH$_2$CH), 6.63 (2H, br d, $C_6H_4NH_2$), 7.04 (1H, t, C_6H_3CO), 7.53 (3H, m, C_6H_3CO and $C_6H_4NH_2$), 7.58 (1H, dd, C_6H_3CO); TSPMS *m*/*z* 562 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{32}FN_7O_5S$: 562.2248, found: 562.2253.

5.69. (2*S*)-(4-Carboxybenzenesulfonyl)amino-3-[3-fluoro-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (50)

A mixture of **47** (20.0 mg, 0.0436 mmol), DMAP (1.10 mg, 0.00872 mmol), and pyridine (0.5 mL) was added 4-(chlorosulfonyl)benzoic acid[21] (9.60 mg, 0.0436 mmol) over 3 h. After an additional 1 h, the mixture was concentrated. The residue was purified by silica gel preparative TLC ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) to give *t*-butyl (2*S*)-(4-carboxybenzenesulfonyl)amino-3-[3-fluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (13.0 mg, 0.0201 mmol, 46%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.23 (9H, s, *t*-Bu), 1.74 (2H, dq, piperidine), 2.11 (2H, br d, piperidine), 2.94 (2H, br t, piperidine), 3.50 (1H, dd, CONHCH_2CH), 3.59 (2H, br d, piperidine), 3.66 (1H, dd, CONHCH_2CH), 3.95 (1H, tt, piperidine), 4.13 (1H, dd, CONHCH$_2$CH), 6.59 (1H, t, pyrimidine), 7.08 (1H, t, C_6H_3CO), 7.48 (1H, dd, C_6H_3CO), 7.49 (1H, dd, C_6H_3CO), 7.83 (2H, d, $C_6H_4CO_2H$), 8.03 (2H, d, $C_6H_4CO_2H$), 8.26 (2H, d, pyrimidine); TSPMS *m*/*z* 643 $(M+H)^+$; $[\alpha]_D^{23}$ +185° (*c* 0.33, MeOH).

The title compound (15.1 mg, 0.0256 mmol, 60% in two steps) was synthesized from this compound following the general procedure for **8**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.71 (2H, br q, piperidine), 1.96 (4H, m, tetrahydropyrimidine and piperidine), 2.96 (2H, br t, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.55 (5H, m, CONHCH_2CH and piperidine), 3.84 (1H, dd, CONHCH$_2$CH), 6.97 (1H, t, C_6H_3CO), 7.29 (1H, dd, C_6H_3CO), 7.45 (1H, dd, C_6H_3CO), 7.76 (2H, d, $C_6H_4CO_2H$), 7.88 (2H, d, $C_6H_4CO_2H$); TSPMS *m*/*z* 591 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{26}H_{31}FN_6O_7S$: 591.2037, found: 591.2013; $[\alpha]_D^{26}$ +36° (*c* 0.76, MeOH/concd NH_4OH = 10:1).

5.70. 3-[3-Fluoro-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-methoxybenzenesulfonyl)amino}propionic acid (51)

The title compound (25.0 mg, 0.0434 mmol, 90% in three steps) was synthesized from **47** and 4-methoxybenzenesulfonyl chloride following the general procedure for **42** (Step 1) and then for **8**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.69 (2H, br dq, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 2.03 (2H, br d, piperidine), 2.90 (2H, br t, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.51 (4H, m, CONHCH_2CH and piperidine), 3.66 (1H, dd, CONHCH_2CH), 3.79 (1H, m, CONHCH$_2$CH), 3.80 (3H, s, C_6H_4O*Me*), 6.92 (2H, br d, C_6H_4OMe), 7.03 (1H, t, C_6H_3CO), 7.46 (1H, dd, C_6H_3CO), 7.53 (1H, dd, C_6H_3CO), 7.75 (2H, br d, C_6H_4OMe); TSPMS *m*/*z* 577 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{26}H_{33}FN_6O_6S$: 577.2245, found: 577.2251; $[\alpha]_D^{23}$ +60° (*c* 0.085, DMSO).

5.71. 3-[3-Fluoro-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-hydroxybenzenesulfonyl)amino}propionic acid (52)

To a solution of *t*-butyl 3-[3-fluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-methoxybenzenesulfonyl)amino}propionate (see experimental for **51**) (66.8 mg, 0.106 mmol) and dichloroethane (5.0 mL), BBr_3 (1.0 M in CH_2Cl_2, 1.10 mL, 1.10 mmol) was added. The mixture was warmed to 40 °C and stirred for 2.5 h. A mixture of 1,4-dioxane (1.0 mL), water (0.2 mL), and Et_3N (1.0 mL) was added to the mixture. The concentrated residue was purified by silica gel preparative TLC ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) and then LH-20 (MeOH) to prepare 3-[3-fluoro-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-(2*S*)-{(4-hydroxybenzenesulfonyl)amino}propionic acid (35.1 mg, 0.0523 mmol, 59%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75 (2H, dq, piperidine), 2.12 (2H, br d, piperidine), 2.94 (2H, br t, piperidine), 3.49 (1H, dd, CONHCH_2CH), 3.59 (2H, br d, piperidine), 3.69 (1H, dd, CONHCH_2CH), 3.86 (1H, dd, CONHCH$_2$CH), 3.95 (1H, tt, piperidine), 6.60 (1H, t, pyrimidine), 6.78 (2H, br d, C_6H_4OH), 7.08 (1H, t, C_6H_3CO), 7.48 (1H, dd, C_6H_3CO), 7.53 (1H, dd, C_6H_3CO), 7.66 (2H, br d, C_6H_4OH), 8.27 (2H, d, pyrimidine); TSPMS *m*/*z* 559 $(M+H)^+$; $[\alpha]_D^{26}$ +36° (*c* 0.46, MeOH).

The title compound (10.7 mg, 0.0125 mmol, 29%) was synthesized from this compound following the general procedure for **8** (Step 2): ^{1}H NMR (400 MHz, CD_3OD) δ 1.69 (2H, dq, piperidine), 1.97 (2H, quintet, tetrahydropyrimidine), 2.03 (2H, br d, piperidine), 2.89 (2H, br t, piperidine), 3.37 (4H, t, tetrahydropyrimidine),

3.51 (4H, m, CONHCH_2CH and piperidine), 3.68 (1H, dd, CONHCH_2CH), 3.78 (1H, dd, CONHCH$_2$CH), 6.80 (2H, br d, C$_6H_4$OH), 7.05 (1H, t, C$_6H_3$CO), 7.51 (1H, dd, C$_6H_3$CO), 7.56 (1H, dd, C$_6H_3$CO), 7.67 (2H, br d, C$_6H_4$OH); ESIMS *m/z* 563 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{31}FN_6O_6S$: 563.2088, found: 563.2085; $[\alpha]_D^{26}$ +108° (*c* 0.54, MeOH/concd NH_4OH = 10:1).

5.72. (2*S*)-(4-Hydroxybenzenesulfonyl)amino-3-[3–hydroxy-4-{4-(1,4,5,6- tetrahydropyrimidin-2-yl-amino)piperidin-1-yl}benzoylamino]propionic acid (53)

The title compound was synthesized from **5e** and 4-methoxybenzenesulfonyl chloride following the general procedure **52**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.70 (2H, br q, piperidine), 1.97 (4H, m, piperidine and tetrahydropyrimidine), 2.71 (2H, br t, piperidine), 3.37 (4H, t, tetrahydropyrimidine), 3.42 (3H, m, piperidine), 3.60 (3H, m, CONHCH_2CH), 6.75 (2H, br d, C$_6H_4$OH), 6.96 (1H, d, C$_6H_3$CO), 7.27 (2H, m, C$_6H_3$CO), 7.63 (2H, d, C$_6H_4$OH); TSPMS *m/z* 561 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_7S$: 561.2131, found: 561.2125; $[\alpha]_D^{23}$ +88° (*c* 1.0, MeOH/concd NH_4OH = 10:1).

5.73. (2*S*)-Amino-3-[4-{4-(1*H*-benzimidazol-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (54)

The ester **41** (30.7 mg, 0.0439 mmol) was dissolved in CH_2Cl_2 (1.0 mL), added TFA (0.1 mL), and stirred at room temperature for 24 h. The solvent was evaporated in vacuo and the residue was purified by silica gel preparative TLC (CH_2Cl_2/EtOH/concd NH_4OH/H_2O = 8:8:1:1), and then LH-20 (MeOH/concd NH_4OH = 10:1) to give **54** (8.70 mg, 0.00206 mmol, 47%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.71 (2H, br dq, piperidine), 2.17 (2H, br d, piperidine), 3.06 (2H, br ddd, piperidine), 3.82 (4H, m, CONHCH_2CH, and piperidine), 3.95 (2H, br d, piperidine), 7.02 (2H, d, C$_6H_4$CO), 7.13 (2H, dd, benzimidazole), 7.29 (2H, dd, benzimidazole), 7.77 (2H, d, C$_6H_4$CO); TSPMS *m/z* 423 $(M+H)^+$; FAB-HMS $(M+H)^+$ calcd for $C_{22}H_{26}N_6O_3$: 423.2145, found: 423.2145; $[\alpha]_D^{23}$ +2.8° (*c* 0.44, MeOH/concd NH_4OH = 1:1).

5.74. (2*S*)-(*N*-Benzenesulfonyl-*N*-methyl)amino-3-[4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (55)

DMF (1.2 mL) was added to **7a** (60.0 mg, 0.103 mmol) to prepare a solution, to which methyl iodide (73.3 mg, 0.516 mmol) and diazabicycloundecene (94.4 mg) were added. The mixture was stirred at room temperature for 16 hr. The reaction solution was concentrated under reduced pressure. The residue was extracted with AcOEt (20 mL). The extract was washed once with distilled water and once with saturated saline in that order, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH = 400:20:1) to prepare the methylated compound (53.1 mg, 0.0894 mmol, 87%).

The compound (52.9 mg, 0.0891 mmol) afforded **55** (14.1 mg, 0.0260 mmol, 29% in two steps) following the general procedure for **1**: ^{1}H NMR (400 MHz, 77% CD_3OD/D_2O) δ 1.60 (2H, br q, piperidine), 1.96 (2H, quintet, tetrahydropyrimidine), 2.02 (2H, br d, piperidine), 2.89 (3H, s, NMe), 2.98 (2H, br t, piperidine), 3.36 (4H, t, tetrahydropyrimidine), 3.55 (1H, m, piperidine), 3.71 (2H, m, CONHCH_2CH), 3.81 (2H, br d, piperidine), 6.59 (1H, t, pyrimidine), 6.99 (2H, d, C$_6H_4$), 7.41 (2H, m, C$_6H_5$), 7.48 (1H, m, C$_6H_5$), 7.66 (2H, d, C$_6H_4$), 7.83 (2H, m, C$_6H_5$); FABMS *m/z* 543 $(M+H)^+$.

5.75. 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 a measurement of absorbance at 415 nm.

5.76. 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 were 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 the absorbance at 405 nm after the cytolysis by SDS. The IC_{50} values were determined graphically from two or more independent experiments.

5.77. 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.

5.78. Aqueous solubility

Aqueous solubility of compounds was determined in water, pH 4 Mcllvaine and pH 8 Mcllvaine 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.

5.79. Single dose rat pharmacokinetic study

Intravenous formulation was prepared by dissolving in DMSO (5 mg/mL) and diluting to a final concentration of 0.5 mg/mL with 5% injectable glucose. Compounds were intravenously administered at 0.5 mg/kg (dosing volume: 1 mL/kg) to non-fasted 9- to 10-week-old male Wistar rats ($n = 2$–3). 0.5 milliliter 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 6000*g* 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-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-inf}, area under concentration curve from hour 0 to infinity; V_{ss}, steady-state distribution volume.

Acknowledgments

The authors thank Shigeko Miki and Takako Miyara for mass spectral analysis, Shuji Ozaki for toxicological studies, and Takashi Watanabe for X-ray crystallography.

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