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Tricyclic pharmacophore-based molecules as novel integrin $\alpha_v\beta_3$ antagonists. Part 1: Design and synthesis of a lead compound exhibiting $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonistic activity

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Abstract—In order to generate novel compounds with integrin $\alpha_v\beta_3$-antagonistic activity together with antiplatelet activity, tricyclic pharmacophore-based molecules were designed and synthesized. Although piperazine-containing compounds initially prepared were selective $\alpha_{IIb}\beta_3$ antagonists, replacement of piperazine with piperidine furnished a potent $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonist. Structure–activity relationship (SAR) studies provided clues for further development of tricyclic pharmacophore-based integrin antagonists.

1. Introduction

Integrins make up a family of heterodimeric transmembrane proteins that mediate essential cell–cell and cell–matrix interactions involved in many physiological and pathological processes.[1,2] Among them, integrin $\alpha_v\beta_3$ is expressed on a variety of cell types, including osteoclasts, leucocytes, vascular smooth muscle cells, and endothelial cells. And integrin $\alpha_v\beta_3$ binds a number of proteins, including vitronectin, fibrinogen, and osteopontin, through recognition of the tripeptide RGD sequence. And antagonists of $\alpha_v\beta_3$ are considered as candidate drugs for treatment of osteoporosis, cancer growth and metastasis, inflammation, diabetic retinopathy, and restenosis.[3] Another member of the family, integrin $\alpha_{IIb}\beta_3$, serves as the final common pathway leading to platelet aggregation via its binding to fibrinogen. Blockade of $\alpha_{IIb}\beta_3$ binding to fibrinogen provides a potent antiplatelet activity, and two $\alpha_{IIb}\beta_3$ antagonists, tirofiban[4] and integrelin,[5] are currently used in the treatment of coronary thrombosis. Our research program has been focused on the functions of integrin $\alpha_v\beta_3$ in vascular smooth muscle cells and leukocytes. Further, Fab fragment of the human-murine monoclonal antibody Abciximab,[6] which binds to the $\alpha_v\beta_3$ receptor and $\alpha_{IIb}\beta_3$ receptor, has clinical efficacy in the treatment of ischemic diseases. In 1996, we started a search for novel integrin $\alpha_v\beta_3$ antagonists with antiplatelet activity as candidate drugs for the treatment of acute ischemic diseases. In this paper, we describe the synthesis of a lead compound exhibiting $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonistic activity, and we present basic SAR data concerning the selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$.

2. Background

When we started our integrin research program, three major pharmaceutical companies had already reported their original $\alpha_v\beta_3$ antagonists, including an aromatic-based antagonist[7–9] and a 1,4-benzodiazepine-type antagonist[10,11] (see Fig. 1). For the molecular design of novel non-peptide integrin $\alpha_v\beta_3$ antagonists, we focused on mimetics of the RGD tripeptide sequence, which is a key recognition site for binding/adhesion. In order to obtain potent antagonists, high affinity between the receptor and antagonist at the molecular level is crucial. Since the spatial arrangement of the ligand molecule is important, we selected a linear, unfused-tricyclic pharmacophore.

Keywords: Non-peptide; Integrin $\alpha_v\beta_3$ antagonist; Integrin $\alpha_{IIb}\beta_3$ antagonist; Acute ischemic disease.
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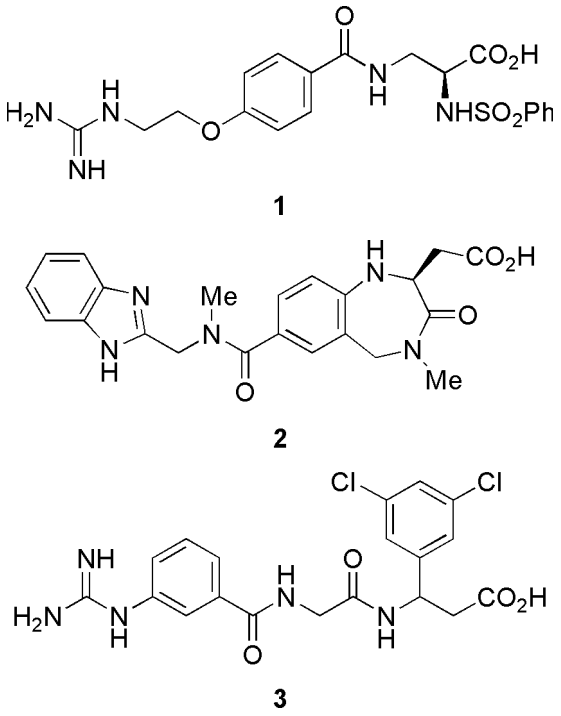
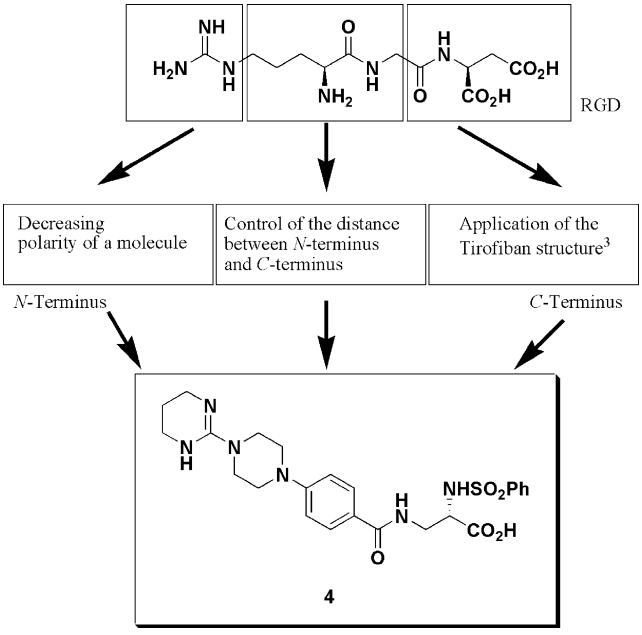


Figure 1. Reported integrin $\alpha_v\beta_3$ antagonists.



Scheme 1. Design of a tricyclic pharmacophore-based molecule.

Thus, we designed compound **4** possessing a cyclic N-terminus and a phenyl piperazine moiety as shown in Scheme 1. At almost at the same time, structurally related antagonists were synthesized by Nicolaou et al.[12]

3. Results and discussion

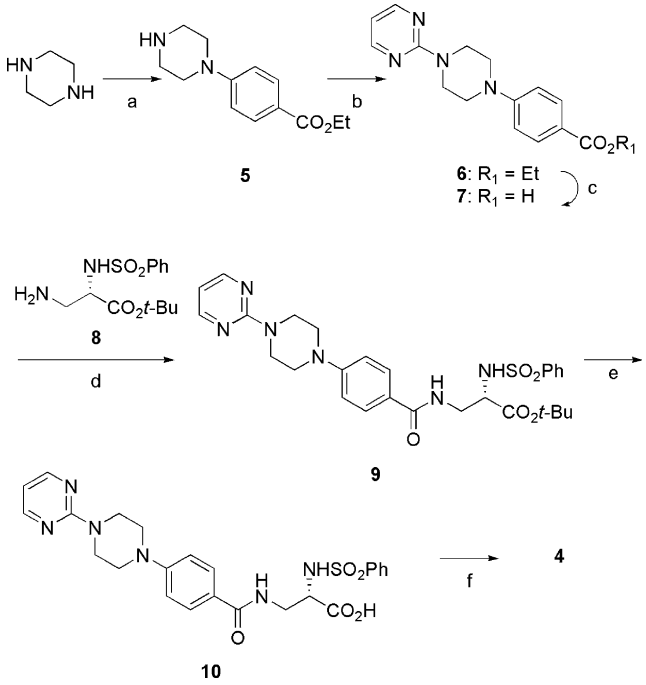
Piperazine was coupled with ethyl 4-fluorobenzoate under heated condition, followed by reaction with 2-bromopyrimidine in the presence of a tertiary amine, to afford a tricyclic moiety (Scheme 2). After basic hydrolysis of the ethyl ester, the C-terminal building block[13] (**8**) was attached to the tricyclic moiety using water-soluble carbodiimide. Deprotection of the *t*-butyl group with trifluoroacetic acid, followed by hydrogenolysis of pyrimidine, furnished the desired compound **4**. This molecule exhibited weak antagonistic activity to $\alpha_v\beta_3$,[14,15] but strong $\alpha_{IIb}\beta_3$-antagonistic activity. Thus, we attempted to enhance the $\alpha_v\beta_3$-antagonistic activity by chemical modification.

Compound **15** was generated through compound **13**, in which the secondary amine at piperazine was protected with a Boc group, as shown in Scheme 3.

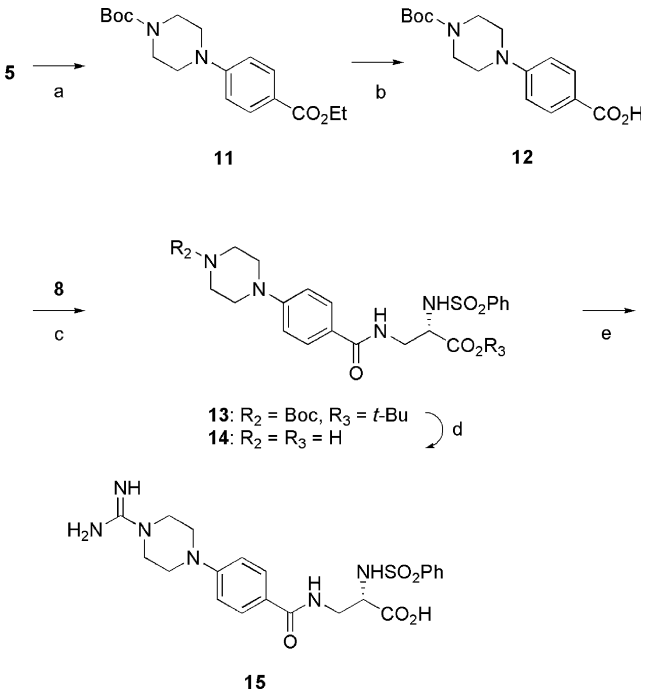
For the synthesis of benzylamine-type compounds (**23**–**25**), reductive amination was utilized (Scheme 4). Thus, ethyl benzoate was converted into benzaldehyde in two steps, and coupling with the C-terminal moiety (**8**) followed by further elaboration gave **23**–**25**.

First, the SAR of the N-terminus and the amide bond was examined, using integrin $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ receptor binding assays (Tables 1 and 2). Regarding the N-terminus, the formimidoyl and benzimidazole derivatives (**15**, **16**) showed moderate inhibition, like the tetrahydropyrimidine analogue (**4**), but we did not proceed with further modification of **16** because of its extremely low water solubility. Transformation of the amide portion failed to improve the activity.

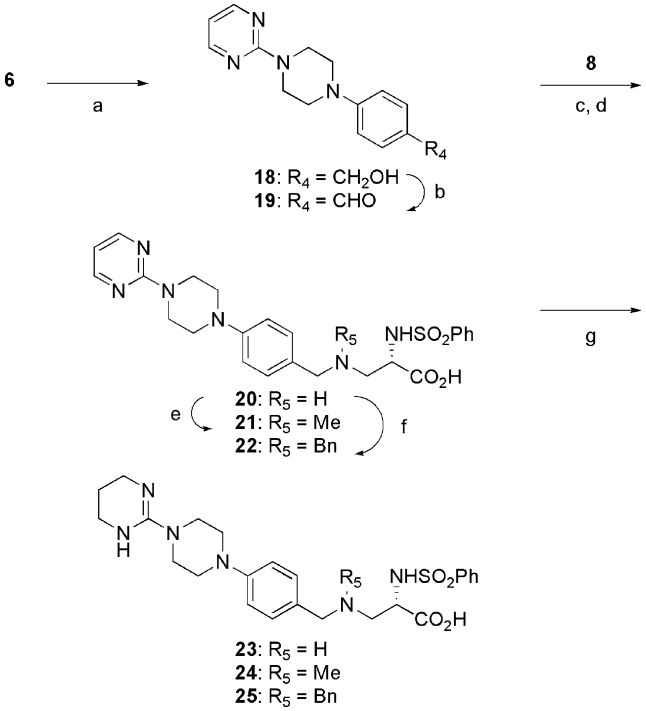
Second, the effect of C-terminal structure was investigated (see Schemes 5 and 6). The importance of an acidic proton at a benzenesulfonylamide group for $\alpha_v\beta_3$-antagonistic activity was confirmed (Table 3).



Scheme 2. Reagents and conditions: (a) Ethyl 4-fluorobenzoate, DMSO, 120 °C, 20 h; (b) 2-bromopyrimidine, *N*,*N*-diisopropylethylamine, DMF, 120 °C, 4.5 h; (c) NaOH, H_2O/MeOH/THF, 40 °C, 5 h; (d) HOBT, *N*-methylmorpholine, EDC, DMF, rt, 24 h; (e) TFA, CH_2Cl_2, rt, 3 h; (f) H_2/Pd-C, 3 atm, HCl/aq AcOH, rt, 4 h.



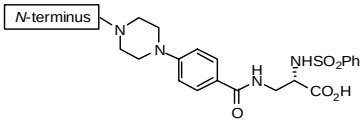
Scheme 3. Reagents and conditions: (a) Boc_2O, NaOH, H_2O/1,4-dioxane, rt, 1 h; (b) NaOH, H_2O/MeOH/THF, 50 °C, 8 h; (c) HOBT, *N*-methylmorpholine, EDC, DMF, rt, 18 h; (d) TFA, H_2O rt, 1 h; (e) 3,5-dimethylpyrazole-1-carboxamide nitrate, pyridine, 90 °C, 20 h.



Scheme 4. Reagents and conditions: (a) DIBAH, CH_2Cl_2 −78 °C, 1 h; (b) MnO_2 AcOEt, rt, 2 h; (c) $NaBH_3$ AcOH, MeOH/CH_2Cl_2 rt, 30 min; (d) TFA, CH_2Cl_2 rt, 16 h; (e) HCHO, AcOH, $NaBH_3CN$, MeOH/CH_2Cl_2 rt, 30 min; (f) PhCHO, AcOH, $NaBH_3CN$, MeOH/CH_2Cl_2 rt, 1.5 h; (g) H_2/Pd-C, 3 atm, AcOH/HCl, rt, 3 h.

Third, substitutions were made in the central aromatic ring. As shown in Table 4, introduction of a halogen atom generally enhanced $\alpha_v\beta_3$-antagonistic activity,

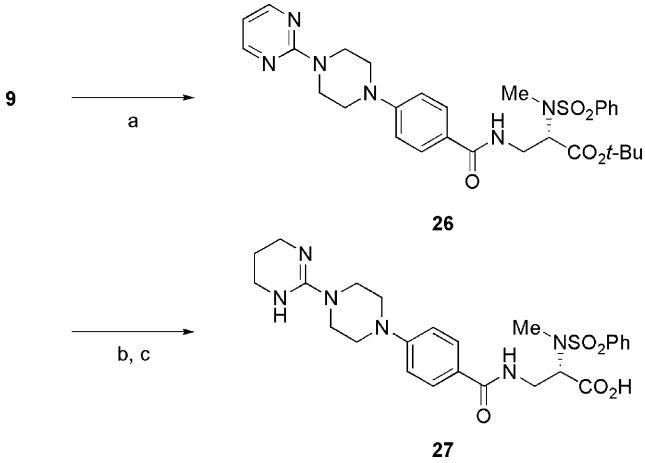
Table 1. Effect of replacement of the N-terminus on receptor binding inhibition



Compound	N-terminus	IC_{50} (nM)		$\alpha_v\beta_3/\alpha_{IIb}\beta_3$
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	
4		160	0.73	0.0046
10		4,700	9.3	0.002
15		220	0.57	0.0026
16		64	1.2	0.019
17	Boc—	4,900	18	0.0037

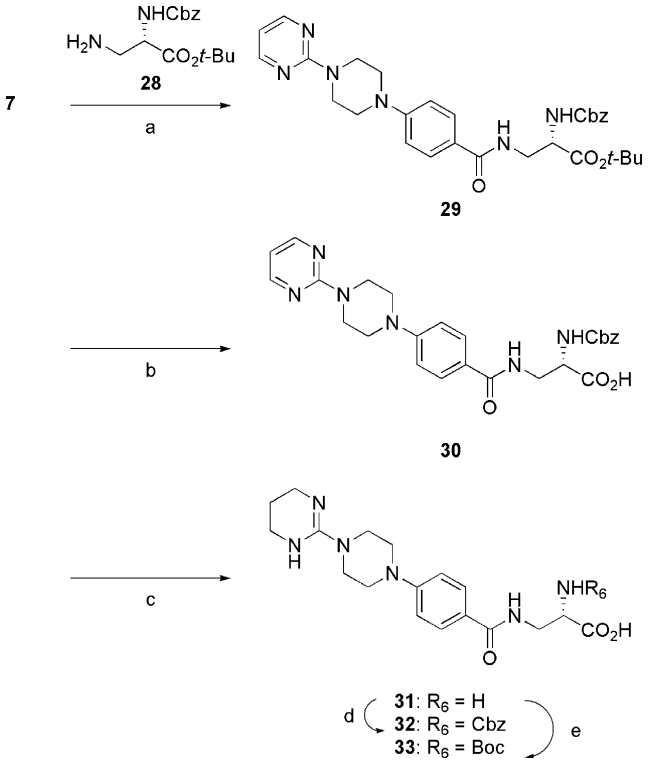
Table 2. Effect of replacement of the amide portion on receptor binding inhibition

Compound	IC_{50} (nM)		$\alpha_v\beta_3/\alpha_{IIb}\beta_3$
	$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	
4	160	0.73	0.0046
23	6,700	76	0.011
24	7,000	10,000	1.4
25	4,900	8,100	1.7



Scheme 5. Reagents and conditions: (a) MeI, DBU, DMF, rt, 16 h; (b) TFA, anisole, CH_2Cl_2, 0 °C, 16 h; (c) H_2/Pd-C, 3 atm, HCl/aq AcOH, rt, 3 h.

but this approach alone failed to yield a dual antagonist. Several antagonists that exhibited potent $\alpha_v\beta_3$ receptor binding inhibition were further evaluated for $\alpha_v\beta_3$-mediated cell adhesion inhibitory activity using vascular smooth muscle cells (VSMC).[16]



Scheme 6. Reagents and conditions: (a) HOBT, *N*-methylmorpholine, EDC, DMF, rt, 24 h; (b) TFA, CH_2Cl_2, rt, 5.5 h; (c)H_2/Pd-C, 3 atm, HCl/aq AcOH, rt, 4 h; (d) CbzCl, K_2CO_3, acetone/H_2O, rt, 6.5 h; (e) Boc_2O NaOH, H_2O, rt, 2 h.

Finally, piperazine was replaced as shown in Table 5. Although introduction of a methyl group into the piperazine ring increased $\alpha_v\beta_3$-antagonistic activity, replacement of piperazine with 4-aminopiperidine drastically enhanced the $\alpha_v\beta_3$ activity, providing compound **47** with $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonistic activity as the first example in this series. This dual activity is expected to exhibit clinical efficacy as well as tirofiban,[17] which is used as remedy for acute coronary syndrome. Moreover, compound **47** showed strong inhibition of $\alpha_v\beta_3$-mediated cell adhesion.

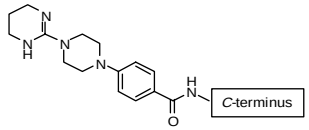
4. Conclusion

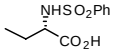
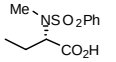
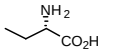
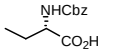
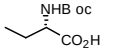
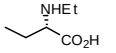
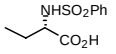
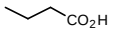
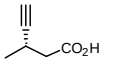
As a design principle for novel $\alpha_v\beta_3$ antagonists, we focused on mimetics of the RGD tripeptide sequence, which is a key recognition site for binding/adhesion. Thus, we designed and synthesized piperazine-based non-peptide integrin $\alpha_v\beta_3$ antagonists with a linear, unfused-tricyclic pharmacophore. Compound **4** exhibited potent $\alpha_{IIb}\beta_3$-antagonistic activity. SAR studies aimed at improving selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$ were conducted, and replacement of piperazine with 4-aminopiperidine afforded compound **47**, with potent $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonistic activity.

5. Experimental

^{1}H NMR spectra were recorded on JNM-LA400 spectrometers with chemical shifts in ppm with the 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

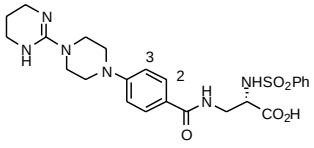
Table 3. Effect of replacement of the C-terminus on receptor binding inhibition



Compound	C-terminus	IC_{50} (nM)		$\alpha_v\beta_3/\alpha_{IIb}\beta_3$
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	
4		160	0.73	0.0046
27		14,000	77	0.0055
31		17,000	1300	0.076
32		35	4.4	0.13
33		120	8.5	0.071
34[a]		49,000	270	0.0055
10[b]		4,700	9.3	0.002
35[b,c]		46,000	7,900	0.17
36[b,d]		180,000	NT	NT

[a] 3-Fluorobenzoyl instead of the central benzoyl.
[b] Pyrimidine at the N-terminus.
[c] Prepared by using β-alanine ethyl ester.
[d] Prepared by using ethyl (3*S*)-ethynyl-3-aminopropionate.

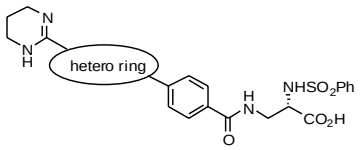
Table 4. Effect of substitution of the central aromatic ring on receptor binding inhibition

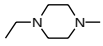
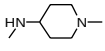
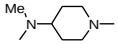


Compound	2 or 3 position	IC_{50} (nM)			$\alpha_v\beta_3/\alpha_{IIb}\beta_3$
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC[b]	
4	Unsubstituted	160	0.73	NT	0.0046
37	3-F	22	1.0	3,500	0.045
38	2-F	31	0.41	5,500	0.013
39	3-Cl	3.6	0.12	4,200	0.033
40	2-Cl	19	0.14	15,000	0.0071
41[a]	3-NO_2	220	1.1	NT	0.0050

[a] Pyrimidine at the N-terminus.
[b] $\alpha_v\beta_3$-mediated cell adhesion assay: vascular smooth muscle cell-vitronectin.

Table 5. Effect of replacement of the hetero ring on receptor binding inhibition



Compound	Hetero rings	IC_{50} (nM)			$\alpha_v\beta_3/\alpha_{IIb}\beta_3$
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC[b]	
4		160	0.73	NT	0.0046
42		67	1.3	3,700	0.019
43		24	0.73	1,600	0.030
44		120	1.7	NT	0.014
45		280	0.77	NT	0.0028
46[a]		270	4.9	NT	0.018
47		1.3	3.1	190	2.4
48		76	0.19	9,000	0.0025

[a] Benzimidazole at the N-terminus.

[b] $\alpha_v\beta_3$-mediated cell adhesion assay: vascular smooth muscle cell-vitronectin.

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. Preparation of compound 4

5.1.1. Compound 5. DMSO (10 ml) was added to piperazine (5.2 g, 60 mmol) to prepare a suspension, to which ethyl 4-fluorobenzoate (2.5 g, 15 mmol) was added at room temperature. The reaction mixture was stirred at 120 °C for 20 h and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 900:100:1) to prepare compound **5** (3.3 g, 94%) as a colorless solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.37 (3H, t, Et), 3.02 (4H, m, piperazine), 3.28 (4H, m, piperazine), 4.32 (2H, q, Et), 6.86 (2H, d, C_6H_4), 7.92 (2H, d, C_6H_4); TSPMS *m*/*z* 235 $(M+H)^+$.

5.1.2. Compound 6. DMF (4.0 ml) was added to compound **5** (114 mg, 0.48 mmol) to prepare a solution, and 2-bromopyrimidine (76 mg, 0.48 mmol) and *N*,*N*-diisopropylethylamine (0.4 ml, 2.3 mmol) were added to the solution at room temperature. The reaction mixture was stirred at 120 °C for 4.5 h and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 950:50:1) to prepare compound **6** (142 mg, 95%) as a colorless solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.37 (3H, t, Et), 3.43 (4H, m, piperazine), 3.99 (4H, m, piperazine), 4.33 (2H, q, Et), 6.54 (1H, t, pyrimidine), 6.90 (2H, d, C_6H_4), 7.94 (2H, d, C_6H_4), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 313 $(M+H)^+$.

5.1.3. Compound 7. MeOH (5.0 ml) and THF (25 ml) were added to compound **6** (127 mg, 0.41 mmol) to prepare a solution, and 1 N NaOH (25 ml) was added to the solution at room temperature. The mixture was stirred at 40 °C for 5.0 h. The reaction mixture was returned to room temperature, neutralized by 1 N HCl (pH 7), and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOH/H_2O/concd NH_4OH = 8:8:1:1) to prepare compound **7** (128 mg, 100%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) δ**:** 3.35 (4H, m, piperazine), 3.95 (4H, m, piperazine), 6.60 (1H, t, pyrimidine), 6.97 (2H, d, C_6H_4), 7.87 (2H, d, C_6H_4), 8.33 (2H, d, pyrimidine); TSPMS *m*/*z* 285 $(M+H)^+$.

5.1.4. Compound 9. DMF (60 ml) was added to compound **7** (734 mg, 2.6 mmol) to prepare a solution. Compound **8** (860 mg, 2.9 mmol) was added to the solution. Further, 1-hydroxybenzotriazole (523 mg, 3.9 mmol), *N*-methylmorpholine (1.5 ml, 14 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (758 mg, 4.0 mmol) were added thereto, and the mixture was stirred at room temperature for 24 h. An aqueous $NaHCO_3$ solution (500 ml) was added to stop the reaction, and the mixture was extracted with CH_2Cl_2 (500 ml). The organic layer was dried over anhydrous Mg_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate = 1:2) to prepare compound **9** (1.0 g, 68%) as a colorless solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.27 (9H, s, *t*-Bu), 3.39 (4H, m, piperazine), 3.57 (1H, m, CONHCH_2CH), 3.89 (2H, m, CONHCH_2CH), 3.99 (4H, m, piperazine), 6.53 (1H, t, pyrimidine), 6.93 (2H, d, C_6H_4), 7.49 (2H, m, C_6H_5), 7.57 (1H, m, C_6H_5), 7.73 (2H, d, C_6H_4), 7.86 (2H, m, C_6H_5), 8.33 (2H, d, pyrimidine); TSPMS *m*/*z* 567 $(M+H)^+$.

5.1.5. Compound 10. CH_2Cl_2 (4.0 ml) was added to compound **9** (51 mg, 0.090 mmol) to prepare a solution. Trifluoroacetic acid (2.0 ml) was added at room temperature to the solution. The reaction mixture was stirred at that temperature for 3.0 h and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (CH_2Cl_2/EtOH/H_2O/concd NH_4OH = 8:8:1:1) and Sephadex LH-20 chromatography (MeOH) to prepare compound **10** (46 mg, 100%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) δ: 3.30 (4H, m, piperazine), 3.46 (1H, dd, CONHCH_2CH), 3.57 (1H, dd, CONHCH_2CH), 3.64 (1H, dd, CONHCH_2CH), 3.87 (4H, m, piperazine), 6.52 (1H, t, pyrimidine), 6.92 (2H, d, C_6H_4), 7.36 (2H, m, C_6H_5), 7.42 (1H, m, C_6H_5), 7.63 (2H, d, C_6H_4), 7.75 (2H, m, C_6H_5), 8.25 (2H, d, pyrimidine); FAB-HRMS $(M+H)^+$ calcd for $C_{24}H_{26}N_6O_5S$ 511.1764, found 511.1759; $[\alpha]_D^{25}$ +63° (*c* 0.055, MeOH).

5.1.6. Compound 4. Acetic acid (100 ml) and concentrated hydrochloric acid (10 ml) were added to compound **10** (378 mg, 0.74 mmol) to prepare a solution. 10% Pd/C (200 mg) was added to the solution, and the mixture was vigorously shaken at room temperature for 4.0 h under a hydrogen pressure of 3 atm. The insolubles were filtered and then washed twice with water. The filtrate was combined with the washings, followed by concentration under reduced pressure. The residue was purified by preparative thin-layer chromatography (CH_2Cl_2/EtOH/H_2O/concd NH_4OH = 8:8:1:1) and Sephadex LH-20 chromatography (MeOH) to prepare compound **4** (198 mg, 52%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) δ: 1.95 (2H, quintet, J = 5.8, tetrahydropyrimidine), 3.37 (4H, m, piperazine), 3.40 (4H, t, J = 5.8, tetrahydropyrimidine), 3.52 (4H, m, piperazine), 3.57 (1H, dd, J = 8.0, 13.2, $CONHCH_2CH$), 3.64 (1H, dd, J = 5.0, 13.2, $CONHCH_2CH$), 3.72 (1H, dd, J = 5.0, 8.0, $CONHCH_2CH$), 6.91 (2H, d, J = 9.0, C_6H_4), 7.47 (2H, m, C_6H_5), 7.53 (1H, m, C_6H_5), 7.72 (2H, d, J = 9.0, C_6H_4), 7.85 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{24}H_{30}N_6O_5S$ 515.2077, found 515.2076; $[\alpha]_D^{25}$ +112° (c 0.048, MeOH).

5.2. Preparation of compound 15

5.2.1. Compound 12. 1,4-Dioxane (10 ml) and H_2O (5.0 ml) were added to compound **5** (500 mg, 2.1 mmol) to prepare a solution. 1 N NaOH (3.0 ml) and di-*t*-butyl dicarbonate (465 mg, 2.1 mmol) were added to the solution at room temperature. After 1.0 h stirring, the reaction mixture was concentrated under reduced pressure to prepare compound **11**.

THF (20 ml) and MeOH (5.0 ml) were added to compound **11** to prepare a solution. 1 N NaOH (20 ml) was added to the solution, and the mixture was stirred at 50 °C for 8.0 h. The reaction mixture was returned to room temperature, neutralized by 1 N HCl (pH 7), and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOH/H_2O/concd NH_4OH = 8:8:1:1) to prepare compound **12** (400 mg, 62%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) δ: 1.48 (9H, s, *t*-Bu), 3.26 (4H, br t, piperazine), 3.57 (4H, br t, piperazine), 6.94 (2H, d, C_6H_4), 7.87 (2H, d, C_6H_4); TSPMS m/z 307 $(M+H)^+$.

5.2.2. Compound 14. DMF (2.0 ml) was added to compound **12** (63 mg, 0.21 mmol) to prepare a solution. Compound **8** (61 mg, 0.20 mmol) was added to the solution. Further, 1-hydroxybenzotriazole (42 mg, 0.31 mmol), *N*-methylmorpholine (0.50 ml, 4.5 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (81 mg, 0.42 mmol) were added thereto, and the mixture was stirred at room temperature for 18 h. An aqueous $NaHCO_3$ solution (20 ml) was added to stop the reaction, and the mixture was extracted with CH_2Cl_2 (20 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate = 2:3) to prepare compound **13** as a colorless solid.

Trifluoroacetic acid (2.0 ml) and H_2O were added to compound **13** (31 mg, 0.053 mmol) to prepare a solution, and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOH/H_2O/concd NH_4OH = 8:8:1:1) and Sephadex LH-20 chromatography (MeOH/concd NH_4OH = 8:1) to prepare compound **14** (7.9 mg, 34%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) δ: 3.30 (5H, m, piperazine, $CONHCH_2CH$), 3.45 (4H, t, piperazine), 3.62 (1H, dd, $CONHCH_2CH$), 4.04 (1H, dd, $CONHCH_2CH$), 6.94 (2H, d, C_6H_4), 7.21 (3H, m, C_6H_5), 7.40 (2H, d, C_6H_4), 7.61 (2H, d, C_6H_5); TSPMS m/z 433 $(M+H)^+$.

5.2.3. Compound 15. Pyridine (10 ml) was added to compound **14** (23 mg, 0.052 mmol) and 3,5-dimethylpyrazole-1-carboxamidine nitrate (22 mg, 0.11 mmol). The reaction mixture was stirred at 90 °C for 20 h and then returned to room temperature. It was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOH/H_2O/concd NH_4OH = 8:8:1:1) and Sephadex G-10 chromatography (0.05 N HCl) to prepare compound **15** hydrochloride (5.3 mg, 17%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) (hydrochloride salt) δ: 3.49 (1H, dd, $CONHCH_2CH$), 3.51 (4H, m, piperazine), 3.71 (4H, m, piperazine), 3.72 (1H, dd, $CONHCH_2CH$), 4.18 (1H, dd, $CONHCH_2CH$), 7.06 (2H, d, C_6H_4), 7.43 (2H, m, C_6H_5), 7.50 (1H, m, C_6H_5), 7.73 (2H, d, C_6H_4), 7.82 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{21}H_{26}N_6O_5S$ 475.1764, found 475.1766; $[\alpha]_D^{25}$ +45° (c 0.26, MeOH).

5.3. Preparation of compound 16

5.3.1. Ethyl 4-{4-(1*H*-benzimidazol-2-yl)piperazin-1-yl}benzoate. DMF (2.0 ml) was added to compound **5** (93 mg, 0.40 mmol) to prepare a solution, and 2-chlorobenzimidazole (37 mg, 0.24 mmol) was added to the solution. The mixture was stirred at 140 °C for 28 h and cooled to room temperature, and water (20 ml) and CH_2Cl_2 (20 ml) were added thereto. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate = 1:2) to prepare the title compound (37 mg, 44%) as a colorless solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.38 (3H, t, Et), 3.44 (4H, m, piperazine), 3.73 (4H, m, piperazine), 4.34 (2H, q, Et), 6.88 (2H, br d, C_6H_4), 7.09 (2H, dd, benzimidazole), 7.34 (2H, m, benzimidazole), 7.95 (2H, br d, C_6H_4); TSPMS m/z 351 $(M+H)^+$.

5.3.2. 4-{4-(1*H*-Benzimidazol-2-yl)piperazin-1-yl}benzoic acid. The title compound was prepared from ethyl 4-{4-(1*H*-benzimidazol-2-yl)piperazin-1-yl}benzoate by the same procedure as employed for compound **7** as a colorless solid. Yield: 20 mg, 92%; ^1H NMR (400 MHz, CD_3OD) δ: 3.38 (4H, m, piperazine), 3.60 (4H, m, piperazine), 6.93 (4H, m, benzimidazole and C_6H_4), 7.17 (2H, dd, benzimidazole), 7.80 (2H, d, C_6H_4); TSPMS m/z 323 $(M+H)^+$.

5.3.3. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(1*H*-benzimidazol-2-yl)piperazin-1-yl}benzoylamino]propionate. The title compound was prepared from 4-{4-(1*H*-benzimidazol-2-yl)piperazin-1-yl}benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 25 mg, 92%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.21 (9H, s, *t*-Bu), 3.21 (4H, m, piperazine), 3.50 (1H, m, $CONHCH_2CH$), 3.64 (4H, m, piperazine), 3.86 (2H, m, $CONHCH_2CH$), 6.82 (2H, d, C_6H_4), 7.03 (2H, dd, benzimidazole), 7.30 (2H, m, benzimidazole), 7.42 (2H, m, C_6H_5), 7.50 (1H, m, C_6H_5), 7.66 (2H, d, C_6H_4), 7.77 (2H, d, C_6H_5); FABMS *m*/*z* 605 $(M+H)^+$.

5.3.4. Compound 16. CH_2Cl_2 (2.0 ml) was added to *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(1*H*-benzimidazol-2-yl)piperazin-1-yl}benzoylamino]propionate (10 mg, 0.018 mmol) to prepare a solution. Trifluoroacetic acid (0.20 ml) was added at room temperature to the solution, and the mixture was stirred at that temperature for 8 h before the reaction solution was concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography on silica gel ($EtOH/H_2O$/concd NH_4OH = 8:1:1) and then purified by Sephadex LH-20 (MeOH/concd NH_4OH = 9:1) to prepare the compound **16** (4.5 mg, 47%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ: 3.21 (4H, m, piperazine), 3.47 (1H, dd, $CONHCH_2CH$), 3.57 (1H, dd, $CONHCH_2CH$), 3.60 (4H, m, piperazine), 3.64 (1H, dd, $CONHCH_2CH$), 6.92 (2H, dd, benzimidazole), 6.96 (2H, d, C_6H_4), 7.17 (2H, dd, benzimidazole), 7.37 (2H, m, C_6H_5), 7.43 (1H, br t, C_6H_5), 7.65 (2H, d, C_6H_4), 7.76 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{27}H_{28}N_6O_5S$ 549.1920, found 549.1929; $[\alpha]_D^{25}$ +89° (*c* 0.068, MeOH).

5.3.5. Compound 17. To a solution of compound **14** (86 mg, 0.20 mmol) in 1,4-dioxane/H_2O (2.0 ml/1.0 ml), 1 N NaOH (1.0 ml) and di-*t*-butyl dicarbonate (57 mg, 0.26 mmol) were added at room temperature. After 1.5 h stirring, the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CH_2Cl_2/EtOH/H_2O$/concd NH_4OH = 8:8:1:1) and Sephadex LH-20 chromatography (MeOH) to prepare compound **17** (85 mg, 80%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.48 (9H, s, *t*-Bu), 3.29 (4H, m, piperazine), 3.53 (1H, dd, $CONHCH_2CH$), 3.57 (4H, m, piperazine), 3.66 (1H, dd, $CONHCH_2CH$), 3.91 (1H, dd, $CONHCH_2CH$), 6.98 (2H, d, C_6H_4), 7.44 (2H, m, C_6H_5), 7.51 (1H, m, C_6H_5), 7.69 (2H, d, C_6H_4), 7.85 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{32}N_4O_7S$ 533.2070, found 533.2061.

5.4. Preparation of compound 23

5.4.1. Compound 20. Compound **6** (200 mg, 0.64 mmol) was dissolved in CH_2Cl_2 (5.6 ml), and 1 M toluene solution of diisobutyl aluminum hydride (1.6 ml) was added dropwise to the solution under cooling at −78 °C over a period of 10 min. The reaction was allowed to proceed at that temperature for 1 h. MeOH (0.8 ml) was added thereto at that temperature and heated to room temperature. CH_2Cl_2 (50 ml) and a saturated aqueous Rochelle salt solution (50 ml) were added thereto, followed by vigorous stirring at room temperature for 15 min. The organic layer was separated, washed twice with saturated saline (50 ml), dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure to prepare compound **18** (158 mg).

Compound **18** (143 mg) was dissolved in ethyl acetate (14 ml), and active manganese dioxide (358 mg) was added to the solution. The mixture was stirred vigorously at room temperature for 2 h. The insolubles were filtered and then washed twice with MeOH. The filtrate was combined with the washings, followed by concentration under reduced pressure to prepare compound **19** (142 mg). 75% $MeOH/CH_2Cl_2$ (14 ml) was added to compound **19** and compound **8** hydrochloride (142 mg, 0.43 mmol) to dissolve them in the $MeOH/CH_2Cl_2$. The reaction solution was adjusted to pH 3–4 by the addition of a small amount of acetic acid. Sodium cyanoborohydride (150 mg) was added thereto, followed by a reaction at room temperature for 30 min. The reaction solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH = 1000:30:1) to prepare *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzylamino]propionate (288 mg) as a colorless solid.

CH_2Cl_2 (6.0 ml) was added to *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-yl)piperazin-1-yl}-benzylamino]propionate (269 mg, 0.49 mmol) to prepare a solution, and anisole (0.26 ml) was added to the solution. The mixture was cooled to 0 °C. Trifluoroacetic acid (6.0 ml) was added at that temperature to the mixture, and the reaction was allowed to proceed at room temperature for 16 h. The reaction solution was concentrated under reduced pressure. The residue was subjected to azeotropic distillation twice with toluene, followed by purification by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH = 90:20:1) to prepare compound **20** (224 mg, 0.45 mmol) as a colorless solid; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 1:1) δ: 3.18 (1H, dd, $ArCH_2NHCH_2$), 3.28 (1H, dd, $ArCH_2NHCH_2$), 3.33 (4H, m, piperazine), 3.58 (1H, dd, $PhSO_2NHCH$), 3.98 (4H, m, piperazine), 4.08 (2H, ABq, $ArCH_2$), 6.62 (1H, t, pyrimidine), 7.02 (2H, d, C_6H_4), 7.34 (2H, d, C_6H_4), 7.54 (2H, m, $SO_2C_6H_5$), 7.62 (1H, m, $SO_2C_6H_5$), 7.88 (2H, m, $SO_2C_6H_5$), 8.35 (2H, d, pyrimidine); TSPMS (neg.) *m*/*z* 495 $(M-H)^-$; $[\alpha]_D^{25}$ +99° (*c* 0.40, $MeOH/CHCl_3$ = 1:1).

5.4.2. Compound 23. Compound **23** was prepared from compound **20** by the same procedure as employed for compound **4** as a colorless solid. Yield: 27 mg, 61%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.90 (2H, quintet, tetrahydropyrimidine), 2.97 (2H, m, $ArCH_2NHCH_2$), 3.21 (4H, m, piperazine), 3.34 (4H, t, tetrahydropyrimidine), 3.46 (4H, m, piperazine), 3.60 (1H, dd, $PhSO_2NHCH$), 3.86 (2H, ABq, $ArCH_2$), 6.92 (2H, d, C_6H_4), 7.26 (2H, d, C_6H_4), 7.47 (2H, m, $SO_2C_6H_5$), 7.54 (1H, m, $SO_2C_6H_5$), 7.82 (2H, m, $SO_2C_6H_5$); TSPMS *m*/*z* 501 $(M+H)^+$; $[\alpha]_D^{25}$ +74° (*c* 1.0, MeOH).

5.5. Preparation of compound 24

5.5.1. Compound 21. 50% MeOH/CH_2Cl_2 (12 ml) was added to compound **20** (80 mg, 0.16 mmol) to prepare a solution. A 37% aqueous formaldehyde solution (131 mg) was added to the solution, and the reaction solution was then adjusted to pH 3–4 by the addition of a minor amount of acetic acid. Sodium cyanoborohydride (60 mg) was added thereto, and a reaction was allowed to proceed at room temperature for 30 min. The reaction solution was concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH = 90:15:1) to prepare compound **21** (66 mg, 81%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$ = 1:1) δ: 2.70 (3H, s, NMe), 3.22 (1H, dd, $ArCH_2NMeCH_2$), 3.34 (4H, m, piperazine), 3.64 (1H, dd, $PhSO_2NHCH$), 3.98 (4H, m, piperazine), 4.13 (2H, ABq, $ArCH_2$), 6.62 (1H, t, pyrimidine), 7.02 (2H, d, C_6H_4), 7.34 (2H, d, C_6H_4), 7.55 (2H, m, $SO_2C_6H_5$), 7.62 (1H, m, $SO_2C_6H_5$), 7.89 (2H, m, $SO_2C_6H_5$), 8.35 (2H, d, pyrimidine); TSPMS *m*/*z* 511 $(M+H)^+$; $[\alpha]_D^{25}$ +77° (*c* 0.8, $CHCl_3$).

5.5.2. Compound 24. Compound **24** was prepared from compound **21** by the same procedure as employed for compound **4** as a colorless solid. Yield: 33 mg, 40%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.90 (2H, quintet, tetrahydropyrimidine), 2.21 (3H, s, NMe), 2.76 (2H, m, $ArCH_2NMeCH_2$), 3.19 (4H, m, piperazine), 3.35 (4H, t, tetrahydropyrimidine), 3.48 (4H, m, piperazine), 3.56 (1H, br d, $ArCH_2$), 3.66 (1H, br d, $ArCH_2$), 3.70 (1H, dd, $PhSO_2NHCH$), 6.88 (2H, d, C_6H_4), 7.20 (2H, d, C_6H_4), 7.46 (2H, m, $SO_2C_6H_5$), 7.53 (1H, m, $SO_2C_6H_5$), 7.83 (2H, m, $SO_2C_6H_5$); TSPMS *m*/*z* 515 $(M+H)^+$; $[\alpha]_D^{25}$ +27° (*c* 1.0, MeOH).

5.6. Preparation of compound 25

5.6.1. Compound 22. Compound **22** was prepared from compound **20** by the similar procedure as employed for compound **21** using 5.0 equiv of benzaldehyde instead of the formaldehyde solution as a colorless solid. Yield: 8.3 mg, 71%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.12 (1H, dd, $ArCH_2NBzlCH_2$), 3.27 (1H, dd, $ArCH_2NBzlCH_2$), 3.32 (4H, m, piperazine), 3.52 (1H, dd, $PhSO_2NHCH$), 3.87 (1H, d, $ArCH_2$), 3.92 (1H, d, $ArCH_2$), 3.99 (1H, d, $ArCH_2$), 3.99 (4H, m, piperazine), 4.03 (1H, d, $ArCH_2$), 6.54 (1H, t, pyrimidine), 6.96 (2H, d, C_6H_4), 7.27 (2H, d, C_6H_4), 7.40 (5H, m, $C_6H_5CH_2N$), 7.48 (2H, br t, $SO_2C_6H_5$), 7.55 (1H, br t, $SO_2C_6H_5$), 7.77 (2H, br d, $SO_2C_6H_5$), 8.35 (2H, d, pyrimidine); FABMS (+NaI) *m*/*z* 609 $(M+Na)^+$; $[\alpha]_D^{25}$ +118° (*c* 0.8, $CHCl_3$).

5.6.2. Compound 25. The title compound was prepared from compound **22** by the same procedure as employed for compound **4**. Yield: 5.1 mg, 34%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.92 (2H, m, tetrahydropyrimidine), 2.92 (1H, dd, $ArCH_2NBzlCH_2$), 3.06 (1H, dd, $ArCH_2NBzlCH_2$), 3.24 (4H, m, piperazine), 3.36 (4H, t, tetrahydropyrimidine), 3.49 (4H, m, piperazine), 3.54 (1H, dd, $PhSO_2NHCH$), 3.83 (2H, ABq, $ArCH_2$), 3.91 (2H, ABq, $ArCH_2$), 6.92 (2H, d, C_6H_4), 7.25 (2H, d, C_6H_4), 7.33 (5H, m, $C_6H_5CH_2N$), 7.46 (2H, m, $SO_2C_6H_5$), 7.54 (1H, m, $SO_2C_6H_5$), 7.75 (2H, m, $SO_2C_6H_5$); FABMS *m*/*z* 591 $(M+H)^+$; $[\alpha]_D^{25}$ +36° (*c* 0.5, MeOH).

5.7. Preparation of compound 27

5.7.1. Compound 26. The compound **9** (40 mg, 0.071 mmol) was dissolved in DMF (0.80 ml). Methyl iodide (50 mg, 0.35 mmol) and 1,8-diazabicyclo[5.4.0]-7-undecene (65 mg, 0.43 mmol) were added to the solution. The reaction was allowed to proceed at room temperature for 16 h. The reaction solution was concentrated under reduced pressure. The residue was extracted with ethyl acetate (8.0 ml), followed by washing with water and saturated saline in that order. The organic layer was dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH = 200:10:1) to prepare compound **26** (41 mg, 100%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (9H, s, *t*-Bu), 2.89 (3H, s, NMe), 3.39 (4H, m, piperazine), 3.76 (1H, ddd, $CONHCH_2CH$), 3.88 (1H, ddd, $CONHCH_2CH$), 3.99 (4H, m, piperazine), 4.73 (1H, dd, $CONHCH_2CH$), 6.54 (1H, t, pyrimidine), 6.95 (2H, d, C_6H_4), 7.50 (2H, m, C_6H_5), 7.57 (1H, m, C_6H_5), 7.77 (2H, d, C_6H_4), 7.87 (2H, m, C_6H_5), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 581 $(M+H)^+$.

5.7.2. 2-(*N*-Benzenesulfonyl-*N*-methylamino)-3-[4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. The title compound was prepared from compound **26** by the same procedure as employed for compound **10**. Yield: 25 mg, 33% as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ: 2.90 (3H, s, NMe), 3.39 (4H, m, piperazine), 3.71 (1H, dd, $CONHCH_2CH$), 3.79 (1H, dd, $CONHCH_2CH$), 3.96 (4H, m, piperazine), 6.61 (1H, t, pyrimidine), 7.01 (2H, d, C_6H_4), 7.38 (2H, m, C_6H_5), 7.46 (1H, m, C_6H_5), 7.70 (2H, d, C_6H_4), 7.81 (2H, m, C_6H_5), 8.35 (2H, d, pyrimidine); TSPMS *m*/*z* 525 $(M+H)^+$.

5.7.3. Compound 27. Compound **27** was prepared from 2-(*N*-benzenesulfonyl-*N*-methylamino)-3-[4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid by the same procedure as employed for compound **4** as a colorless solid. Yield: 13 mg, 53%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.96 (2H, quintet, tetrahydropyrimidine), 2.89 (3H, s, NMe), 3.38–3.43 (8H, m, tetrahydropyrimidine and piperazine), 3.56 (4H, m, piperazine), 3.72 (2H, d, $CONHCH_2CH$), 4.72 (1H, t, $CONHCH_2CH$), 6.95 (2H, d, C_6H_4), 7.38 (2H, m, C_6H_5), 7.46 (1H, m, C_6H_5), 7.70 (2H, d, C_6H_4), 7.85 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_5S$ 529.2233, found 529.2223.

5.8. Preparation of compound 31

5.8.1. Compound 29. DMF (6.0 ml) was added to compound **7** (101 mg, 0.35 mmol) to prepare a solution, and compound **28** (107 mg, 0.35 mmol) was added to the solution. Further, 1-hydroxybenzotriazole (63 mg, 0.47 mmol), *N*-methylmorpholine (0.70 ml), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochlo-

ride (121 mg, 0.63 mmol) were added to the mixture, and the mixture was stirred at room temperature for 24 h. An aqueous $NaHCO_3$ solution (60 ml) was added to the reaction solution to stop the reaction, and CH_2Cl_2 (60 ml) was added thereto. The organic layer was then separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 950:50:1) to prepare compound **29** (53 mg, 27%) as a colorless solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.46 (9H, s, *t*-Bu), 3.40 (4H, m, piperazine), 3.81 (2H, m, $CONHCH_2CH$), 4.01 (4H, m, piperazine), 4.45 (1H, m, $CONHCH_2CH$), 5.12 (2H, s, $CH_2C_6H_5$), 6.57 (1H, t, pyrimidine), 6.92 (2H, d, C_6H_4), 7.31 (5H, m, $CH_2C_6H_5$), 7.69 (2H, d, C_6H_4), 8.38 (2H, d, pyrimidine); TSPMS *m*/*z* 561 $(M+H)^+$.

5.8.2. Compound 30. Compound **30** was prepared from compound **29** by the same procedure as employed for compound **10** as a colorless solid. Yield: 13 mg, 17%; ^1H NMR (400 MHz, CD_3OD) δ: 3.29 (4H, t, piperazine), 3.57 (1H, dd, $CONHCH_2CH$), 3.65 (1H, dd, $CONHCH_2CH$), 3.86 (4H, t, piperazine), 4.17 (1H, dd, $CONHCH_2CH$), 4.96 (2H, dd, $CH_2C_6H_5$), 6.52 (1H, t, pyrimidine), 6.91 (2H, d, C_6H_4), 7.19 (5H, m, $CH_2C_6H_5$), 7.62 (2H, d, C_6H_4), 8.25 (2H, d, pyrimidine); TSPMS *m*/*z* 505 $(M+H)^+$; $[\alpha]_D^{25}$ −9.6° (*c* 0.072, MeOH).

5.8.3. Compound 31. Compound **31** was prepared by the same procedure as employed for compound **4** as a colorless solid. Yield: 4.9 mg, 65%; ^1H NMR (400 MHz, CD_3OD) δ: 1.97 (2H, quintet, tetrahydropyrimidine), 3.43 (8H, m, piperazine), 3.56 (4H, m, tetrahydropyrimidine), 3.81 (3H, m, $CONHCH_2CH$), 6.99 (2H, d, C_6H_4), 7.79 (2H, d, C_6H_4); FABMS *m*/*z* 375 $(M+H)^+$; $[\alpha]_D^{25}$ −6.2° (*c* 0.11, MeOH).

5.9. Preparation of compound 32

5.9.1. Compound 32. Acetone (1.0 ml) and water (1.0 ml) were added to the compound **31** (26 mg, 0.069 mmol) to prepare a solution. Potassium carbonate (55 mg) was added to the solution. Benzyloxycarbonyl chloride (0.040 ml) was added to the mixture, and the mixture was stirred for 6.5 h and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography on silica gel (EtOH/H_2O/concd NH_4OH = 6:1:1) and then purified by Sephadex LH-20 (MeOH/concd NH_4OH = 9:1) to prepare compound **32** (2.3 mg, 6.5%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) δ: 1.97 (2H, quintet, tetrahydropyrimidine), 3.40 (8H, m, piperazine and tetrahydropyrimidine), 3.54 (4H, m, piperazine), 3.71 (2H, m, $CONHCH_2CH$), 4.24 (1H, m, $CONHCH_2CH$), 5.05 (2H, dd, $CH_2C_6H_5$), 6.94 (2H, d, C_6H_4), 7.28 (5H, m, $CH_2C_6H_5$), 7.71 (2H, d, C_6H_4); TSPMS *m*/*z* 509 $(M+H)^+$; $[\alpha]_D^{25}$ −3.4° (*c* 0.052, MeOH).

5.10. Preparation of compound 33

5.10.1. Compound 33. A 1 N aqueous NaOH (2.0 ml) was added to the compound **31** (22 mg, 0.059 mmol), and an excess of di-*t*-butyl dicarbonate was added to the solution. The mixture was stirred for 2 h and cooled to room temperature. The mixture was adjusted to pH 8 by the addition of 1 N hydrochloric acid and then concentrated under reduced pressure. The residue was purified by Sephadex LH-20 (MeOH) to prepare compound **33** (12 mg, 43%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) δ: 1.40 (9H, s, *t*-Bu), 1.97 (2H, quintet, tetrahydropyrimidine), 3.41 (8H, m, piperazine and tetrahydropyrimidine), 3.54 (4H, m, piperazine), 3.67 (2H, m, $CONHCH_2CH$), 4.20 (1H, m, $CONHCH_2CH$), 6.95 (2H, d, C_6H_4), 7.74 (2H, d, C_6H_4); FAB-HRMS $(M+H)^+$ calcd for $C_{23}H_{34}N_6O_5$ 475.2669, found 475.2669; $[\alpha]_D^{25}$ +5.7° (*c* 0.58, MeOH).

5.11. Preparation of compound 34

5.11.1. Methyl 3-fluoro-4-(piperazin-1-yl)benzoate. DMSO (1.0 ml) was added to piperazine (344 mg, 4.0 mmol) to prepare a suspension, to which methyl 3,4-difluorobenzoate (367 mg, 2.1 mmol) was added. The mixture was stirred at 80 °C for 4.5 h. The temperature of the system was then returned to room temperature. The reaction solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 900:100:1) to prepare the title compound (392 mg, 78%) as a colorless solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.05 (4H, m, piperazine), 3.17 (4H, m, piperazine), 3.88 (3H, s, CH_3), 6.91 (1H, t, C_6H_3), 7.67 (1H, dd, C_6H_3), 7.75 (1H, dd, C_6H_3); EIMS *m*/*z* 238.

5.11.2. Methyl 3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoate. The title compound was prepared from methyl 3-fluoro-4-(piperazin-1-yl)benzoate by the same procedure as employed for compound **6** as a colorless solid. Yield: 184 mg, 69%; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.27 (4H, m, piperazine), 3.89 (3H, s, CH_3), 4.00 (4H, m, piperazine), 6.54 (1H, t, pyrimidine), 6.94 (1H, t, C_6H_3), 7.70 (1H, dd, C_6H_3), 7.77 (1H, dd, C_6H_3), 8.36 (2H, d, pyrimidine); TSPMS *m*/*z* 317 $(M+H)^+$.

5.11.3. 3-Fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid. The title compound was prepared from methyl 3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoate by the same procedure as employed for compound **7** as a colorless solid. Yield: 150 mg, 85%; ^1H NMR (400 MHz, CD_3OD) δ: 3.26 (4H, m, piperazine), 3.97 (4H, m, piperazine), 6.62 (1H, t, pyrimidine), 7.10 (1H, t, C_6H_3), 7.65 (1H, dd, C_6H_3), 7.77 (1H, dd, C_6H_3), 8.35 (2H, d, pyrimidine); TSPMS *m*/*z* 303 $(M+H)^+$.

5.11.4. *t*-Butyl (2*S*)-benzyloxycarbonylamino-3-[3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoyl amino]propionate. The title compound was prepared from 3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid and compound **28** by the same procedure as employed for compound **29** as a colorless solid. Yield: 420 mg, 76%; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.46 (9H, s, *t*-Bu), 3.22 (4H, br t, piperazine), 3.78 (2H, m, $CONHCH_2CH$), 4.00 (4H, br t, piperazine), 4.45 (1H, m, $CONHCH_2CH$), 5.12 (2H, d, $CH_2C_6H_5$), 6.53 (1H, t, pyrimidine), 6.92 (1H, t, C_6H_3), 7.32 (5H, m, $CH_2C_6H_5$), 7.45 (1H, d, C_6H_3), 7.50 (1H, d, C_6H_3), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 579 $(M+H)^+$; $[\alpha]_D^{25}$ −12° (*c* 0.98, CH_2Cl_2).

5.11.5. *t*-Butyl (2*S*)-ethylamino-3-[3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate. THF (60 ml) and EtOH (30 ml) were added to *t*-butyl (2*S*)-benzyloxycarbonylamino-3-[3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate to (210 mg, 0.36 mmol) to prepare a solution, and 10% Pd/C (210 mg) was added to the solution. The mixture was stirred vigorously in a hydrogen atmosphere at room temperature for 24 h. The insolubles were filtered and then washed twice with THF. The filtrate was combined with the washings, followed by concentration under reduced pressure. The residue was purified by preparative thin-layer chromatography on silica gel (CH_2Cl_2/MeOH = 9:1) to prepare the title compound (56 mg, 33%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.12 (3H, t, Et), 1.47 (9H, s, *t*-Bu), 2.59 (1H, dq, Et), 2.71 (1H, dq, Et), 3.22 (4H, br t, piperazine), 3.34 (1H, dd, $CONHCH_2CH$), 3.44 (1H, ddd, $CONHCH_2CH$), 3.77 (1H, ddd, $CONHCH_2CH$), 4.00 (4H, br t, piperazine), 6.53 (1H, t, pyrimidine), 6.95 (1H, t, C_6H_3), 7.50 (2H, m, C_6H_3), 8.34 (2H, d, pyrimidine); FABMS *m*/*z* 473 $(M+H)^+$; $[\alpha]_D^{25}$ +7.3° (*c* 1.0, CH_2Cl_2).

5.11.6. (2*S*)-Ethylamino-3-[3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. The title compound was prepared from *t*-butyl (2*S*)-ethylamino-3-[3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1- yl}benzoylamino]propionate by the same procedure as employed for compound **10** as a pale yellow solid. Yield: 64 mg; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 (3H, t, Et), 3.25 (6H, m, piperazine and Et), 3.83 (1H, dd, $CONHCH_2CH$), 3.98 (4H, br t, piperazine), 4.07 (1H, dd, $CONHCH_2CH$), 4.20 (1H, dd, $CONHCH_2CH$), 6.66 (1H, t, pyrimidine), 7.11 (1H, t, C_6H_3), 7.61 (1H, dd, C_6H_3); TSPMS *m*/*z* 417 $(M+H)^+$; $[\alpha]_D^{25}$ +1.9° (*c* 0.84, MeOH).

5.11.7. Compound 34. 1,4-Dioxane (10 ml) and water (5.0 ml) were added to (2*S*)-ethylamino-3-[3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid (64 mg) to prepare a solution, and 10% Pd/C (52 mg) was added to the solution. The mixture was vigorously stirred in a hydrogen atmosphere at room temperature for 8.0 h. The insolubles were filtered and then washed twice with 1,4-dioxane. The filtrate was combined with the washings, followed by concentration under reduced pressure, to prepare compound **34** (50 mg, 99%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.29 (3H, t, Et), 1.97 (2H, quintet, tetrahydropyrimidine), 3.06 (2H, q, Et), 3.25 (4H, m, piperazine), 3.42 (4H, br t, tetrahydropyrimidine), 3.55 (4H, br t, piperazine), 3.60 (1H, dd, $CONHCH_2CH$), 3.74 (1H, dd, $CONHCH_2CH$), 3.84 (1H, dd, $CONHCH_2CH$), 7.08 (1H, t, C_6H_3), 7.60 (1H, d, C_6H_3), 7.64 (1H, d, C_6H_3); FAB-HRMS $(M+H)^+$ calcd for $C_{20}H_{29}N_6O_3F$ 421.2363, found 421.2363; $[\alpha]_D^{25}$ +23° (*c* 1.0, MeOH).

5.12. Preparation of compound 35

5.12.1. Ethyl 3-[4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate. DMF (10 ml) was added to compound **7** (97 mg, 0.34 mmol) to prepare a solution, and β-alanine ethyl ester hydrochloride (176 mg) was added to the solution. Further, 1-hydroxybenzotriazole (72 mg), *N*-methylmorpholine (0.18 ml), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (148 mg) were added thereto, and the mixture was stirred at room temperature for 24 h. An aqueous $NaHCO_3$ solution (30 ml) was added to stop the reaction. Ethyl acetate (300 ml) and water (100 ml) were added thereto. The organic layer was then separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 15:1) to prepare the title compound (110 mg, 84%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.27 (3H, t, Et), 2.64 (2H, t, $CONHCH_2CH_2$), 3.38 (4H, br t, piperazine), 3.71 (2H, q, $CONHCH_2CH_2$), 3.99 (4H, m, piperazine), 4.17 (2H, q, Et), 6.54 (1H, t, pyrimidine), 6.93 (2H, d, C_6H_4), 7.70 (2H, d, C_6H_4), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 384 $(M+H)^+$.

5.12.2. Compound 35. MeOH (0.20 ml) and THF (0.60 ml) were added to ethyl 3-[4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate (40 mg, 0.10 mmol) to prepare a solution, and a 1 N aqueous NaOH (0.20 ml) was added to the solution. The mixture was stirred at 60 °C for 5.0 h and cooled to room temperature, and the system was adjusted to pH 7 by the addition of 1 N hydrochloric acid. The resultant precipitate was collected by filtration on a glass filter and then dried to prepare compound **35** (21 mg, 57%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ: 2.60 (2H, t, $CONHCH_2CH_2$), 3.38 (4H, br t, piperazine), 3.60 (2H, t, $CONHCH_2CH_2$), 3.95 (4H, m, piperazine), 6.62 (1H, t, pyrimidine), 7.02 (2H, d, C_6H_4), 7.73 (2H, d, C_6H_4), 8.34 (2H, d, pyrimidine); FAB-HRMS $(M+H)^+$ calcd for $C_{18}H_{21}N_5O_3$ 356.1723, found 356.1710.

5.13. Preparation of compound 36

5.13.1. Ethyl (3*S*)-[4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]pent-4-ynate. DMF (3.0 ml) was added to compound **7** (24 mg, 0.084 mmol) to prepare a solution, and ethyl 3-amino (3*S*)-ethynyl-propionate hydrochloride (21 mg) was added to the solution. Further, 1-hydroxybenzotriazole (23 mg), *N*-methylmorpholine (0.060 ml), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (33 mg) were added to the mixture, and the mixture was stirred at room temperature for 23 h. An aqueous $NaHCO_3$ solution (30 ml) was added to the mixture to stop the reaction, and CH_2Cl_2 (30 ml) was added thereto. The organic layer was then separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate = 3:7) to prepare the title compound (19 mg, 55%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (3H, t, Et), 2.30 (1H, d, C (triple bond) CH), 2.78 (1H, dd, $CONHCHCH_2$), 2.86 (1H, dd, $CONHCHCH_2$), 3.39 (4H, m, piperazine), 3.99 (4H, m, piperazine), 4.22 (2H, q, Et), 5.32 (1H, m, $CONHCHCH_2$), 6.54 (1H, t, pyrimidine), 6.94 (2H, d, C_6H_4), 7.73 (2H, d, C_6H_4), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 408 $(M+H)^+$.

5.13.2. Compound 36. The title compound was prepared from ethyl (3*S*)-[4-{4-(pyrimidin-2-yl)piperazin-1-yl}ben-

zoylamino]pent-4-ynate by the same procedure as employed for compound **35** as a colorless solid. Yield: 4.5 mg, 47%; ^{1}H NMR (400 MHz, CD_3OD) δ: 2.44 (1H, d, C (triple bond) CH), 2.51 (1H, d, CONHCHCH$_2$), 2.53 (1H, d, CONHCHCH$_2$), 3.31 (4H, m, piperazine), 3.86 (4H, m, piperazine), 5.02 (1H, dt, CONHCHCH$_2$), 6.51 (1H, t, pyrimidine), 6.92 (2H, d, C_6H_4), 7.66 (2H, d, C_6H_4), 8.25 (2H, d, pyrimidine); FAB-HRMS $(M+H)^+$ calcd for $C_{20}H_{21}N_5O_3$ 380.1723, found 370.1718.

5.14. Preparation of compound 37

5.14.1. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate. The title compound was prepared from 3-fluoro-4-{4-(pyrimidin-2-yl)-piperazin-1-yl}benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 218 mg, 76%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 3.23 (4H, br t, piperazine), 3.54 (1H, m, CONHCH$_2$CH), 3.90 (2H, m, CONHCH$_2$CH), 4.01 (4H, br t, piperazine), 6.53 (1H, t, pyrimidine), 6.95 (1H, t, C_6H_3), 7.53 (5H, m, C_6H_3 and C_6H_5), 7.85 (2H, m, C_6H_5), 8.33 (2H, d, pyrimidine); TSPMS *m*/*z* 585 $(M+H)^+$.

5.14.2. (2*S*)-Benzenesulfonylamino-3-[3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. CH_2Cl_2 (10 ml) was added to *t*-butyl (2*S*)-benzenesulfonylamino-3-[3-fluoro-4-{4-(pyrimidin -2-yl)piperazin-1-yl}benzoylamino]propionate (212 mg, 0.36 mmol) to prepare a solution. Trifluoroacetic acid (3.0 ml) was added at room temperature to the solution. The mixture was stirred at that temperature for 7 h before the reaction solution was concentrated under reduced pressure to prepare trifluoroacetate of the title compound (258 mg) as a pale yellow solid; ^{1}H NMR (400 MHz, CD_3OD) (as trifluoroacetate) δ: 3.28 (4H, m, piperazine), 3.47 (1H, dd, CONHCH$_2$CH), 3.71 (1H, dd, CONHCH$_2$CH), 4.00 (4H, br t, piperazine), 4.18 (1H, dd, CONHCH$_2$CH), 6.77 (1H, t, pyrimidine), 7.09 (1H, t, C_6H_3), 7.45 (4H, m, C_6H_3 and C_6H_5), 7.53 (1H, dd, C_6H_3), 7.81 (2H, m, C_6H_5), 8.43 (2H, d, pyrimidine); TSPMS *m*/*z* 529 $(M+H)^+$; $[\alpha]_D^{25}$ +27° (*c* 1.0, MeOH).

5.14.3. Compound 37. Compound **37** was prepared from (2*S*)-benzenesulfonylamino-3-[3-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1- yl}benzoylamino]propionic acid the same procedure as employed for compound **4** as a colorless solid. Yield: 44 mg, 23%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.97 (2H, quintet, tetrahydropyrimidine), 3.25 (4H, br t, piperazine), 3.42 (4H, br t, tetrahydropyrimidine), 3.55 (5H, m, piperazine and CONHCH$_2$CH), 3.67 (1H, dd, CONHCH$_2$CH), 3.77 (1H, dd, CONHCH$_2$CH), 7.08 (1H, t, C_6H_3), 7.49 (3H, m, C_6H_5), 7.55 (1H, dd, C_6H_3), 7.60 (1H, dd, C_6H_3), 7.86 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{24}H_{29}N_6O_5SF$ 533.1982, found 533.1981; $[\alpha]_D^{25}$ +24° (*c* 1.0, MeOH).

5.15. Preparation of compound 38

5.15.1. Methyl 2-fluoro-4-(piperazin-1-yl)benzoate. DMSO (1.0 ml) was added to piperazine (544 mg, 6.3 mmol) to prepare a suspension, to which methyl 2,4-difluorobenzoate (368 mg, 2.1 mmol) was added. The mixture was stirred at 80 °C for 1 h and cooled to room temperature. The reaction solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 900:100:1) to prepare the title compound (274 mg, 55%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.00 (4H, m, piperazine), 3.29 (4H, m, piperazine), 3.88 (3H, s, CH_3), 6.51 (1H, dd, C_6H_3), 6.63 (1H, dd, C_6H_3), 7.82 (1H, t, C_6H_3); EIMS *m*/*z* 238.

5.15.2. Methyl 2-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoate. The title compound was prepared from methyl 2-fluoro-4-(piperazin-1-yl)benzoate by the same procedure as employed for compound **6** as a colorless solid. Yield: 120 mg, 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.44 (4H, br t, piperazine), 3.91 (3H, s, CH_3), 3.98 (4H, br t, piperazine), 6.55 (1H, dd, C_6H_3), 6.55 (1H, t, pyrimidine), 6.66 (1H, dd, C_6H_3), 7.85 (1H, t, C_6H_3), 8.35 (2H, d, pyrimidine); TSPMS *m*/*z* 317 $(M+H)^+$.

5.15.3. 2-Fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid. The title compound was prepared from methyl 2-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}-benzoate by the same procedure as employed for compound **7** as a colorless solid. Yield: 60 mg, 54%; ^{1}H NMR (400 MHz, CD_3OD) δ: 3.47 (4H, m, piperazine), 4.11 (4H, m, piperazine), 6.62 (1H, t, pyrimidine), 6.69 (1H, dd, C_6H_3), 6.80 (1H, dd, C_6H_3), 7.81 (1H, t, C_6H_3), 8.35 (2H, d, pyrimidine); TSPMS *m*/*z* 303 $(M+H)^+$.

5.15.4. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[2-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate. The title compound was prepared from 2-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 64 mg, 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 3.42 (4H, br t, piperazine), 3.70 (1H, m, CONHCH$_2$CH), 3.77 (1H, m, CONHCH$_2$CH), 4.11 (5H, m, piperazine and CONHCH$_2$CH), 6.54 (1H, dd, C_6H_3), 6.55 (1H, t, pyrimidine), 6.74 (1H, dd, C_6H_3), 7.45 (2H, m, C_6H_5), 7.52 (1H, m, C_6H_5), 7.83 (2H, m, C_6H_5), 7.95 (1H, t, C_6H_3), 8.35 (2H, d, pyrimidine); FABMS *m*/*z* 585 $(M+H)^+$; $[\alpha]_D^{25}$ +40° (*c* 1.0, CH_2Cl_2).

5.15.5. (2*S*)-Benzenesulfonylamino-3-[2-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. CH_2Cl_2 (3.0 ml) was added to *t*-butyl (2*S*)-benzenesulfonylamino-3-[2-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate (61 mg, 0.10 mmol) to prepare a solution. Trifluoroacetic acid (1.0 ml) was added at room temperature to the solution. The mixture was stirred at that temperature for 2.5 h before the reaction solution was concentrated under reduced pressure to prepare trifluoroacetate of the title compound (69 mg) as a pale yellow solid; ^{1}H NMR (400 MHz, CD_3OD) (as trifluoroacetate) δ: 3.46 (1H, dd, CONHCH$_2$CH), 3.50 (4H, br t, piperazine), 3.77 (1H, dd, CONHCH$_2$CH), 3.99 (4H, br t, piperazine), 4.17 (1H, dd,

$CONHCH_2CH$), 6.71 (1H, dd, C_6H_3), 6.73 (1H, t, pyrimidine), 6.84 (1H, dd, C_6H_3), 7.44 (3H, m, C_6H_5), 7.70 (1H, t, C_6H_3), 7.82 (2H, m, C_6H_5), 8.43 (2H, d, pyrimidine); TSPMS *m*/*z* 529 $(M+H)^+$; $[\alpha]_D^{25}$ +28° (*c* 1.0, MeOH).

5.15.6. Compound 38. The title compound was prepared from (2*S*)-benzenesulfonylamino-3-[2-fluoro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid by the same procedure as employed for compound **4** as a colorless solid. Yield: 22 mg, 34%; ^1H NMR (400 MHz, CD_3OD) (as hydrochloride) δ: 1.97 (2H, quintet, tetrahydropyrimidine), 3.43 (5H, dd, tetrahydropyrimidine and $CONHCH_2CH$), 3.49 (4H, m, piperazine), 3.56 (4H, m, piperazine), 3.78 (1H, dd, $CONHCH_2CH$), 4.18 (1H, dd, $CONHCH_2CH$), 6.69 (1H, dd, C_6H_3), 6.80 (1H, dd, C_6H_3), 7.44 (3H, m, C_6H_5), 7.71 (1H, t, C_6H_3), 7.82 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{24}H_{29}N_6O_5SF$ 533.1982, found 533.1989; $[\alpha]_D^{25}$ +29° (*c* 0.54, MeOH).

5.16. Preparation of compound 39

5.16.1. Methyl 3-chloro-4-(piperazin-1-yl)benzoate. DMSO (15 ml) was added to piperazine (4.5 g, 52 mmol) to prepare a suspension, to which methyl 3-chloro-4-fluorobenzoate (1.1 g, 5.8 mmol) was added. The mixture was stirred at 80 °C for 5.0 h. The temperature of the system was then returned to room temperature. Ethyl acetate (1000 ml) and water (500 ml) were added thereto. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 5:1) to prepare the title compound (905 mg, 61%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) δ: 2.89 (4H, m, piperazine), 3.01 (4H, m, piperazine), 3.78 (3H, s, CH_3), 7.05 (1H, d, C_6H_3), 7.79 (1H, dd, C_6H_3), 7.86 (1H, d, C_6H_3); TSPMS *m*/*z* 255 $(M+H)^+$.

5.16.2. Methyl 3-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoate. The title compound was prepared from methyl 3-chloro-4-(piperazin-1-yl)benzoate by the same procedure as employed for compound **6** as a colorless solid. Yield: 1.0 g, 86%; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.20 (4H, br t, piperazine), 3.90 (3H, s, CH_3), 4.01 (4H, br t, piperazine), 6.53 (1H, t, pyrimidine), 7.04 (1H, d, C_6H_3), 7.90 (1H, dd, C_6H_3), 8.05 (1H, d, C_6H_3), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 333 $(M+H)^+$.

5.16.3. 3-Chloro-4-{4-(pyrimidin-2-yl)-piperazin-1-yl}benzoic acid. The title compound was prepared from methyl 3-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoate by the same procedure as employed for compound **7** as a colorless solid. Yield: 878 mg, 92%; ^1H NMR (400 MHz, CD_3OD) δ: 3.18 (4H, br t, piperazine), 3.97 (4H, br t, piperazine), 6.61 (1H, t, pyrimidine), 7.18 (1H, d, C_6H_3), 7.90 (1H, dd, C_6H_3), 7.98 (1H, d, C_6H_3), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 319 $(M+H)^+$.

5.16.4. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[3-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate. The title compound was prepared from 3-chloro-4-{4-(pyrimidin-2-yl)-piperazin-1-yl}benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 368 mg, 90%; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 3.18 (4H, br t, piperazine), 3.57 (1H, m, $CONHCH_2CH$), 3.90 (2H, m, $CONHCH_2CH$), 4.02 (4H, br t, piperazine), 6.53 (1H, t, pyrimidine), 7.06 (1H, d, C_6H_3), 7.51 (2H, m, C_6H_5), 7.58 (1H, m, C_6H_5), 7.66 (1H, dd, C_6H_3), 7.86 (3H, d, C_6H_5 and C_6H_3), 8.53 (2H, d, pyrimidine); TSPMS *m*/*z* 601 $(M+H)^+$; $[\alpha]_D^{25}$ +58° (*c* 1.0, CH_2Cl_2).

5.16.5. (2*S*)-Benzenesulfonylamino-3-[3-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. CH_2Cl_2 (25 ml) was added to *t*-butyl (2*S*)-benzenesulfonylamino-3-[3-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate (308 mg, 0.51 mmol) to prepare a solution. Trifluoroacetic acid (10 ml) was added at room temperature to the solution. The mixture was stirred at that temperature for 5.0 h before the reaction solution was concentrated under reduced pressure to prepare trifluoroacetate of the title compound (340 mg) as a pale yellow solid; ^1H NMR (400 MHz, CD_3OD) (as trifluoroacetate) δ: 3.22 (4H, br t, piperazine), 3.47 (1H, dd, $CONHCH_2CH$), 3.71 (1H, dd, $CONHCH_2CH$), 4.00 (4H, br t, piperazine), 4.19 (1H, dd, $CONHCH_2CH$), 6.74 (1H, t, pyrimidine), 7.18 (1H, d, C_6H_3), 7.44 (3H, m, C_6H_5), 7.68 (1H, dd, C_6H_3), 7.79 (1H, d, C_6H_3), 7.81 (2H, m, C_6H_5), 8.43 (2H, d, pyrimidine); TSPMS *m*/*z* 545 $(M+H)^+$; $[\alpha]_D^{25}$ +12° (*c* 0.95, MeOH).

5.16.6. Compound 39. Compound **39** was prepared from (2*S*)-benzenesulfonylamino-3-[3-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid by the same procedure as employed for compound **4** as a colorless solid. Yield: 69 mg, 85%; ^1H NMR (400 MHz, CD_3OD) δ: 1.97 (2H, quintet, tetrahydropyrimidine), 3.17 (4H, br t, piperazine), 3.42 (4H, br t, tetrahydropyrimidine), 3.55 (5H, dd, piperazine and $CONHCH_2CH$), 3.70 (1H, m, $CONHCH_2CH$), 3.76 (1H, m, $CONHCH_2CH$), 7.16 (1H, d, C_6H_3), 7.47 (2H, m, C_6H_5), 7.53 (1H, m, C_6H_5), 7.75 (1H, dd, C_6H_3), 7.85 (3H, d, C_6H_5 and C_6H_3); FAB-HRMS $(M+H)^+$ calcd for $C_{24}H_{29}N_6O_5SCl$ 549.1687, found 549.1696; $[\alpha]_D^{25}$ +66° (*c* 0.53, MeOH).

5.17. Preparation of compound 40

5.17.1. Methyl 2-chloro-4-(piperazin-1-yl)benzoate. DMSO (15 ml) was added to piperazine (4.5 g, 52 mmol) to prepare a suspension, to which methyl 2-chloro-4-fluorobenzoate (3.3 g, 17 mmol) was added. The mixture was stirred at 80 °C for 1.0 h. The temperature of the system was then returned to room temperature. Ethyl acetate (1000 ml) and water (500 ml) were added thereto. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to prepare the title compound (3.7 g, 83%) as a colorless solid; ^1H NMR (400 MHz, CD_3OD) δ: 2.83 (4H, m, piperazine), 3.18 (4H, m, piperazine), 3.73 (3H, s, CH_3), 6.76 (1H, dd, C_6H_3), 6.85 (1H, d, C_6H_3), 7.70 (1H, d, C_6H_3); TSPMS *m*/*z* 255 $(M+H)^+$.

5.17.2. Methyl 2-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoate. The title compound was from methyl 2-chloro-4-(piperazin-1-yl)benzoate prepared by the same procedure as employed for compound **6** as a colorless solid. Yield: 2.7 g, 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.42 (4H, br t, piperazine), 3.88 (3H, s, CH_3), 3.98 (4H, br t, piperazine), 6.55 (1H, t, pyrimidine), 6.77 (1H, dd, C_6H_3), 6.91 (1H, d, C_6H_3), 7.86 (1H, d, C_6H_3), 8.35 (2H, d, pyrimidine); TSPMS *m*/*z* 333 $(M+H)^+$.

5.17.3. 2-Chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid. The title compound was prepared from methyl 2-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoate by the same procedure as employed for compound **7** as a colorless solid. Yield: 1.4 g, 98%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.42 (4H, br t, piperazine), 3.85 (4H, br t, piperazine), 6.66 (1H, t, pyrimidine), 6.94 (1H, dd, C_6H_3), 6.99 (1H, d, C_6H_3), 7.77 (1H, d, C_6H_3), 8.39 (2H, d, pyrimidine); TSPMS *m*/*z* 319 $(M+H)^+$.

5.17.4. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[2-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate. The title compound was prepared from 2-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 355 mg, 92%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 3.37 (4H, br t, piperazine), 3.73 (1H, dd, $CONHCH_2CH$), 3.81 (1H, ddd, $CONHCH_2CH$), 3.98 (5H, m, piperazine and $CONHCH_2CH$), 6.55 (1H, t, pyrimidine), 6.85 (2H, m, C_6H_3), 7.49 (2H, m, C_6H_5), 7.57 (1H, m, C_6H_5), 7.73 (1H, d, C_6H_3), 7.86 (2H, m, C_6H_5), 8.35 (2H, d, pyrimidine); TSPMS *m*/*z* 601 $(M+H)^+$; $[\alpha]_D^{25}$ +38° (*c* 1.0, CH_2Cl_2).

5.17.5. (2*S*)-Benzenesulfonylamino-3-[2-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. CH_2Cl_2 (25 ml) was added to *t*-butyl (2*S*)-benzenesulfonylamino-3-[2-chloro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate (289 mg, 0.48 mmol) to prepare a solution. Trifluoroacetic acid (10 ml) was added at room temperature to the solution. The mixture was stirred at that temperature for 6.0 h before the reaction solution was concentrated under reduced pressure to prepare trifluoroacetate of the title compound (321 mg) as a pale yellow solid; ^{1}H NMR (400 MHz, CD_3OD)(as trifluoroacetate) δ: 3.41 (4H, br t, piperazine), 3.48 (1H, dd, $CONHCH_2CH$), 3.71 (1H, dd, $CONHCH_2CH$), 3.97 (4H, m, piperazine), 4.18 (1H, dd, $CONHCH_2CH$), 6.72 (1H, t, pyrimidine), 6.94 (1H, dd, C_6H_3), 7.00 (1H, d, C_6H_3), 7.45 (1H, d, C_6H_3), 7.50 (2H, m, C_6H_5), 7.57 (1H, m, C_6H_5), 7.86 (2H, m, C_6H_5), 8.41 (2H, d, pyrimidine); TSPMS *m*/*z* 545 $(M+H)^+$; $[\alpha]_D^{25}$ +18° (*c* 0.56, DMSO).

5.17.6. Compound 40. Compound **40** was prepared from (2*S*)-benzenesulfonylamino-3-[2-chloro-4-{4-(pyrimidin-2-yl)piperazin-1- yl}benzoylamino]propionic acid by the same procedure as employed for compound **4** as a colorless solid. Yield: 58 mg, 69%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.97 (2H, quintet, tetrahydropyrimidine), 3.41 (8H, br t, piperazine and tetrahydropyrimidine), 3.54 (4H, m, piperazine), 3.59 (1H, dd, $CONHCH_2CH$), 3.68 (1H, dd, $CONHCH_2CH$), 3.75 (1H dd, $CONHCH_2CH$), 6.90 (1H, dd, C_6H_3), 6.96 (1H, d, C_6H_3), 7.55 (4H, d, C_6H_5 and C_6H_3), 7.87 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{24}H_{29}N_6O_5SCl$ 549.1687, found 549.1695; $[\alpha]_D^{25}$ +68° (*c* 0.37, MeOH).

5.18. Preparation of compound 41

5.18.1. 3-Nitro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid. DMSO (10 ml) was added to 1-(2-pyrimidyl)piperazine dihydrochloride (2.3 g, 9.7 mmol) to prepare a suspension. 4-Fluoro-3-nitrobenzoic acid (1.9 g, 10 mmol) was added to the suspension. *N*,*N*-Diisopropylethylamine (1.0 ml) was added thereto. The mixture was stirred at 120 °C for 17 h and cooled to room temperature. Ethyl acetate (3000 ml) and water (1000 ml) were added thereto. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to prepare the title compound (570 mg, 18%) as a yellow solid; ^{1}H NMR (400 MHz, CD_3OD) δ: 3.27 (4H, br t, piperazine), 3.96 (4H, br t, piperazine), 6.63 (1H, t, pyrimidine), 7.32 (1H, d, C_6H_3), 8.11 (1H, dd, C_6H_3), 8.35 (2H, d, pyrimidine), 8.39 (1H, d, C_6H_3); FABMS *m*/*z* 330 $(M+H)^+$.

5.18.2. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[3-nitro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate. The title compound was prepared from 3-nitro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid by the same procedure as employed for compound **9** as a yellow solid. Yield: 493 mg, 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (9H, s, *t*-Bu), 3.26 (4H, br t, piperazine), 3.53 (1H, m, $CONHCH_2CH$), 3.91 (1H, m, $CONHCH_2CH$), 3.96 (1H, d, $CONHCH_2CH$), 4.01 (4H, br t, piperazine), 6.55 (1H, t, pyrimidine), 7.15 (1H, d, C_6H_3), 7.51 (2H, m, C_6H_5), 7.59 (1H, m, C_6H_5), 7.86 (2H, m, C_6H_5), 7.93 (1H, dd, C_6H_3), 8.31 (1H, d, C_6H_3), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 612 $(M+H)^+$; $[\alpha]_D^{25}$ +58° (*c* 1.0, CH_2Cl_2).

5.18.3. Compound 41. CH_2Cl_2 (25 ml) was added to *t*-butyl (2*S*)-benzenesulfonylamino-3-[3-nitro-4-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate (233 mg, 0.38 mmol) to prepare a solution, and trifluoroacetic acid (10 ml) was added at room temperature to the solution. The mixture was stirred at that temperature for 5.5 h before the reaction solution was concentrated under reduced pressure to prepare trifluoroacetate of compound **41** (259 mg, 76%) as a yellow solid; ^{1}H NMR (400 MHz, CD_3OD) (as trifluoroacetate) δ: 3.29 (4H, br t, piperazine), 3.47 (1H, dd, $CONHCH_2CH$), 3.73 (1H, dd, $CONHCH_2CH$), 3.98 (4H, br t, piperazine), 4.20 (1H, d, $CONHCH_2CH$), 6.72 (1H, t, pyrimidine), 7.32 (1H, d, C_6H_3), 7.43 (3H, m, C_6H_5), 7.81 (2H, m, C_6H_5), 7.92 (1H, dd, C_6H_3), 8.18 (1H, d, C_6H_3), 8.41 (2H, d, pyrimidine); FAB-HRMS $(M+H)^+$ calcd for $C_{24}H_{25}N_7O_7S$ 556.1614, found 556.1607; $[\alpha]_D^{25}$ +25° (*c* 1.1, MeOH).

5.19. Preparation of compound 42

5.19.1. Ethyl 4-{(3*R*)-methyl-(piperazin-1-yl)}benzoate. DMSO (2.0 ml) was added to (2*R*)-methylpiperazine

(295 mg, 2.9 mmol) to prepare a suspension, to which ethyl 4-fluorobenzoate (420 mg, 2.5 mmol) was added. The mixture was stirred at 120 °C for 6.0 h. The temperature of the system was then returned to room temperature, and the reaction solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 900:100:1) to prepare the title compound (410 mg, 66%) as a pale yellow solid; ^1H NMR (400 MHz, CD_3OD) δ: 0.90 (3H, d, CH_3), 1.26 (3H, t, Et), 2.36 (1H, m, piperazine), 2.70 (1H, m, piperazine), 2.81 (2H, m, piperazine), 2.98 (1H, m, piperazine), 3.66 (2H, m, piperazine), 4.20 (2H, q, Et), 6.86 (2H, d, C_6H_4), 7.77 (2H, d, C_6H_4); FABMS *m*/*z* 249 $(M+H)^+$.

5.19.2. Ethyl 4-{(3*R*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoate. The title compound was prepared from ethyl 4-{(3*R*)-methyl-(piperazin-1-yl)}benzoate by the same procedure as employed for compound **6** as a pale yellow solid. Yield: 290 mg, 63%; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.31 (3H, d, CH_3), 1.37 (3H, t, Et), 3.08 (1H, ddd, piperazine), 3.28 (1H, dd, piperazine), 3.50 (1H, ddd, piperazine), 3.68 (1H, dt, piperazine), 3.79 (1H, ddt, piperazine), 4.33 (2H, q, Et), 4.52 (1H, dt, piperazine), 4.95 (1H, m, piperazine), 6.52 (1H, t, pyrimidine), 6.86 (2H, d, C_6H_4), 7.94 (2H, d, C_6H_4), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 327 $(M+H)^+$.

5.19.3. 4-{(3*R*)-Methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid. The title compound was prepared from ethyl 4-{(3*R*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoate by the same procedure as employed for compound **7** as a colorless solid. Yield: 52 mg, 47%; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.31 (3H, d, CH_3), 3.14 (1H, ddd, piperazine), 3.34 (1H, dd, piperazine), 3.53 (1H, ddd, piperazine), 3.77 (1H, m, piperazine), 3.83 (1H, m, piperazine), 4.51 (1H, dt, piperazine), 4.95 (1H, m, piperazine), 6.54 (1H, t, pyrimidine), 6.88 (2H, d, C_6H_4), 8.00 (2H, d, C_6H_4), 8.36 (2H, d, pyrimidine); EIMS *m*/*z* 298.

5.19.4. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{(3*R*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoyl amino]-propionate. The title compound was prepared from 4-{(3*R*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 85 mg, 86%; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.28 (9H, s, *t*-Bu), 1.32 (3H, d, CH_3), 3.03 (1H, dt, piperazine), 3.23 (1H, dd, piperazine), 3.47 (1H, ddd, piperazine), 3.59 (1H, ddd, $CONHCH_2CH$), 3.65 (1H, m, piperazine), 3.76 (1H, m, piperazine), 3.89 (1H, ddd, $CONHCH_2CH$), 3.94 (1H, dt, $CONHCH_2CH$), 4.54 (1H, dt, piperazine), 4.96 (1H, m, piperazine), 6.53 (1H, t, pyrimidine), 6.88 (1H, d, C_6H_4), 7.49 (2H, m, C_6H_5), 7.56 (1H, m, C_6H_5), 7.73 (2H, d, C_6H_4), 7.86 (2H, m, C_6H_5), 8.35 (2H, d, pyrimidine); FABMS *m*/*z* 581 $(M+H)^+$.

5.19.5. (2*S*)-Benzenesulfonylamino-3-[4-{(3*R*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. The title compound was prepared from *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{(3*R*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoyl amino]propionate by the same procedure as employed for compound **10** as a colorless solid. Yield: 30 mg, 77%; ^1H NMR (400 MHz, CD_3OD) δ: 1.21 (3H, d, CH_3), 2.88 (1H, dt, piperazine), 3.08 (1H, dd, piperazine), 3.36 (1H, ddd, piperazine), 3.47 (1H, dd, $CONHCH_2CH$), 3.56 (1H, dd, $CONHCH_2CH$), 3.63 (1H, dd, $CONHCH_2CH$), 3.68 (1H, m, piperazine), 3.76 (1H, m, piperazine), 4.42 (1H, dt, piperazine), 4.79 (1H, m, piperazine), 6.51 (1H, t, pyrimidine), 6.89 (2H, d, C_6H_4), 7.35 (2H, m, C_6H_5), 7.43 (1H, m, C_6H_5), 7.63 (2H, d, C_6H_4), 7.76 (2H, m, C_6H_5), 8.25 (2H, d, pyrimidine); TSPMS *m*/*z* 525 $(M+H)^+$; $[\alpha]_D^{25}$ +55° (*c* 0.073, MeOH).

5.19.6. Compound 42. Compound **42** was prepared from (2*S*)-benzenesulfonylamino-3-[4-{(3*R*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid by the same procedure as employed for compound **4** as a colorless solid. Yield: 2.5 mg, 25%; ^1H NMR (400 MHz, CD_3OD) δ: 1.34 (3H, d, CH_3), 1.96 (2H, quintet, tetrahydropyrimidine), 2.99 (1H, dt, piperazine), 3.18 (1H, dd, piperazine), 3.42 (4H, br t, tetrahydropyrimidine), 3.47 (1H, m, piperazine), 3.57 (1H, dd, $CONHCH_2CH$), 3.60 (1H, m, piperazine), 3.65 (1H, dd, $CONHCH_2CH$), 3.71 (1H, dd, $CONHCH_2CH$), 3.73 (1H, m, piperazine), 3.80 (1H, m, piperazine), 4.09 (1H, m, piperazine), 6.95 (2H, d, C_6H_4), 7.48 (2H, m, C_6H_5), 7.55 (1H, m, C_6H_5), 7.74 (2H, d, C_6H_4), 7.86 (2H, m, C_6H_5); FABMS *m*/*z* 529 $(M+H)^+$; $[\alpha]_D^{25}$ +81° (*c* 0.060, MeOH).

5.20. Preparation of compound 43

5.20.1. Ethyl 4-{(3*S*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoate. DMSO (2.0 ml) was added to (2*S*)-methylpiperazine (310 mg, 3.1 mmol) to prepare a suspension, to which ethyl 4-fluorobenzoate (530 mg, 3.2 mmol) was added. The mixture was stirred at 120 °C for 21 h. The temperature of the system was then returned to room temperature, and the reaction solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 1900:100:1) to prepare ethyl 4-{(3*S*)-methyl-(piperazin-1-yl)}benzoate (450 mg) as a pale yellow solid.

DMF (10 ml) was added to ethyl 4-{(3*S*)-methyl-(piperazin-1-yl)}benzoate (450 mg, 1.8 mmol) to prepare a solution, and 2-bromopyrimidine (480 mg, 3.0 mmol) was added to the solution. *N*,*N*-Diisopropylethylamine (1.5 ml) was added thereto. The mixture was stirred at 120 °C for 15 h and cooled to room temperature, followed by concentration under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate = 1:2) to prepare the title compound (330 mg, 56%) as a pale yellow solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.31 (3H, d, CH_3), 1.37 (3H, t, Et), 3.08 (1H, ddd, piperazine), 3.28 (1H, dd, piperazine), 3.50 (1H, ddd, piperazine), 3.68 (1H, dt, piperazine), 3.79 (1H, ddt, piperazine), 4.33 (2H, q, Et), 4.52 (1H, dt, piperazine), 4.95 (1H, m, piperazine), 6.52 (1H, t, pyrimidine), 6.86 (2H, d, C_6H_4), 7.94 (2H, d, C_6H_4), 8.34 (2H, d, pyrimidine); FABMS *m*/*z* 327 $(M+H)^+$.

5.20.2. 4-{(3*S*)-Methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid. The title compound was prepared from ethyl 4-{(3*S*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoate by

the same procedure as employed for compound **7** as a colorless solid. Yield: 155 mg, 81%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.19 (3H, d, CH_3), 2.92 (1H, ddd, piperazine), 3.12 (1H, dd, piperazine), 3.37 (1H, ddd, piperazine), 3.71 (1H, dt, piperazine), 3.78 (1H, ddt, piperazine), 4.40 (1H, dt, piperazine), 4.81 (1H, m, piperazine), 6.51 (1H, t, pyrimidine), 6.86 (2H, d, C_6H_4), 7.79 (2H, d, C_6H_4), 8.25 (2H, d, pyrimidine); TSPMS *m*/*z* 299 $(M+H)^+$.

5.20.3. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{(3*S*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoyl amino]propionate. The title compound was prepared from 4-{(3*S*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 98 mg, 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28 (9H, s, *t*-Bu), 1.33 (3H, d, CH_3), 3.04 (1H, dt, piperazine), 3.23 (1H, dd, piperazine), 3.47 (1H, ddd, piperazine), 3.58 (1H, ddd, $CONHCH_2CH$), 3.66 (1H, br d, piperazine), 3.77 (1H, br d, piperazine), 3.91 (2H, m, $CONHCH_2CH$), 4.53 (1H, dt, piperazine), 4.96 (1H, m, piperazine), 6.53 (1H, t, pyrimidine), 6.89 (2H, d, C_6H_4), 7.49 (2H, t, C_6H_5), 7.57 (1H, t, C_6H_5), 7.73 (2H, d, C_6H_4), 7.86 (2H, br d, C_6H_5), 8.35 (2H, d, pyrimidine); APCIMS *m*/*z* 581 $(M+H)^+$.

5.20.4. (2*S*)-Benzenesulfonylamino-3-[4-{(3*S*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. The title compound was prepared from *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{(3*S*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoyl amino]propionate by the same procedure as employed for compound **10** as a colorless solid. Yield: 39 mg, 44%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.21 (3H, d, CH_3), 2.89 (1H, dt, piperazine), 3.08 (1H, dd, piperazine), 3.38 (2H, m, piperazine and $CONHCH_2CH$), 3.59 (1H, dd, $CONHCH_2CH$), 3.68 (1H, br d, $CONHCH_2CH$), 3.76 (1H, br d, piperazine), 3.95 (1H, m, piperazine), 4.41 (1H, dt, piperazine), 4.82 (1H, m, piperazine), 6.51 (1H, t, pyrimidine), 6.87 (2H, d, C_6H_4), 7.33 (2H, t, C_6H_5), 7.40 (1H, t, C_6H_5), 7.58 (2H, d, C_6H_4), 7.74 (2H, d, C_6H_5), 8.25 (2H, d, pyrimidine); TSPMS *m*/*z* 525 $(M+H)^+$; $[\alpha]_D^{25}$ +36° (*c* 0.073, MeOH).

5.20.5. Compound 43. Compound **43** was prepared from (2*S*)-benzenesulfonylamino-3-[4-{(3*S*)-methyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid by the same procedure as employed for compound **4** as a colorless solid. Yield: 2.2 mg, 32%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.33 (3H, d, CH_3), 1.96 (2H, quintet, tetrahydropyrimidine), 2.97 (1H, ddd, piperazine), 3.16 (1H, dd, piperazine), 3.42 (4H, br t, tetrahydropyrimidine), 3.45 (1H, m, piperazine), 3.58 (1H, dd, $CONHCH_2CH$), 3.59 (1H, m, piperazine), 3.65 (1H, dd, $CONHCH_2CH$), 3.70 (1H, m, piperazine), 3.72 (1H, dd, $CONHCH_2CH$), 3.79 (1H, m, piperazine), 4.09 (1H, m, piperazine), 6.93 (2H, d, C_6H_4), 7.48 (2H, m, C_6H_5), 7.55 (1H, m, C_6H_5), 7.73 (2H, d, C_6H_4), 7.86 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_5S$ 529.2233, found 529.2239; $[\alpha]_D^{25}$ +96° (*c* 0.085, MeOH).

5.21. Preparation of compound 44

5.21.1. Ethyl 4-{*cis*-3,5-dimethyl-(piperazin-1-yl)}benzoate. DMSO (10 ml) was added to *cis*-2,6-dimethylpiperazine (3.6 g, 32 mmol) to prepare a suspension, to which ethyl 4-fluorobenzoate (2.5 g, 15 mmol) was added. The mixture was stirred at 80 °C for 24 h. The temperature of the system was then returned to room temperature and water (100 ml) was added thereto. The mixture was extracted three times with ethyl acetate (100 ml). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 900:100:1) to prepare the title compound (1.5 g, 38%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.16 (6H, d, CH_3), 1.35 (3H, t, Et), 2.38 (2H, dd, piperazine), 2.95 (2H, m, piperazine), 3.78 (2H, dd, piperazine), 4.29 (2H, q, Et), 6.95 (2H, d, C_6H_4), 7.86 (2H, d, C_6H_4); TSPMS *m*/*z* 263 $(M+H)^+$.

5.21.2. Ethyl 4-{*cis*-3,5-dimethyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoate. The title compound was prepared from ethyl 4-{*cis*-3,5-dimethyl-(piperazin-1-yl)}benzoate by the same procedure as employed for compound **6** as a colorless solid. Yield: 26 mg, 4.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 (6H, d, CH_3), 1.38 (3H, t, Et), 3.15 (2H, dd, piperazine), 3.74 (2H, dd, piperazine), 4.34 (2H, q, Et), 4.89 (2H, m, piperazine), 6.53 (1H, t, pyrimidine), 6.92 (2H, d, C_6H_4), 7.95 (2H, d, C_6H_4), 8.36 (2H, d, pyrimidine); ESIMS *m*/*z* 341 $(M+H)^+$.

5.21.3. 4-{*cis*-3,5-Dimethyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid. The title compound was prepared from ethyl 4-{*cis*-3,5-dimethyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoate by the same procedure as employed for compound **7** as a colorless solid. Yield: 22 mg, 92%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.33 (6H, d, CH_3), 3.11 (2H, dd, piperazine), 3.89 (2H, d, piperazine), 4.87 (2H, m, piperazine), 6.61 (1H, t, pyrimidine), 7.02 (2H, d, C_6H_4), 7.90 (2H, d, C_6H_4), 8.36 (2H, d, pyrimidine); ESIMS *m*/*z* 313 $(M+H)^+$.

5.21.4. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{*cis*-3,5-dimethyl-4-(pyrimidin-2-yl)piperazin-1-yl} benzoylamino]propionate. The title compound was prepared from 4-{*cis*-3,5-dimethyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 25 mg, 66%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 1.38 (6H, d, CH_3), 3.13 (2H, dd, piperazine), 3.56 (1H, m, $CONHCH_2CH$), 3.70 (2H, d, piperazine), 3.91 (2H, m, $CONHCH_2CH$), 4.89 (2H, m, piperazine), 6.52 (1H, t, pyrimidine), 6.96 (2H, d, C_6H_4), 7.50 (2H, m, C_6H_5), 7.58 (1H, m, C_6H_5), 7.74 (2H, d, C_6H_4), 7.87 (2H, m, C_6H_5), 8.37 (2H, d, pyrimidine); TSPMS *m*/*z* 595 $(M+H)^+$.

5.21.5. (2*S*)-Benzenesulfonylamino-3-[4-{*cis*-3,5-dimethyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. CH_2Cl_2 (2.0 ml) was added to *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{*cis*-3,5-dimethyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate (24 mg, 0.040 mmol) to prepare a solution. Trifluoroacetic acid (1.0 ml) was added at room temperature to the solution. The mixture was stirred at that temperature for 6.0 h before the reac-

tion solution was concentrated under reduced pressure to prepare the trifluoroacetate of title compound (25 mg) as a yellow solid; ^{1}H NMR (400 MHz, CD_3OD) (as trifluoroacetate) δ: 1.37 (6H, d, CH_3), 3.11 (2H, m, piperazine), 3.48 (1H, dd, $CONHCH_2CH$), 3.70 (1H, dd, $CONHCH_2CH$), 3.85 (2H, br d, piperazine), 4.17 (1H, dd, CONHCH2CH), 4.84 (2H, m, piperazine), 6.71 (1H, t, pyrimidine), 7.02 (2H, d, C_6H_4), 7.42 (2H, m, C_6H_5), 7.49 (1H, m, C_6H_5), 7.67 (2H, d, C_6H_4), 7.82 (2H, m, C_6H_5), 8.43 (2H, d, pyrimidine); TSPMS *m/z* 539 $(M+H)^+$; $[\alpha]_D^{25}$ +46° (*c* 0.072, MeOH).

5.21.6. Compound 44. Compound **44** was prepared from (2*S*)-benzenesulfonylamino-3-[4-{*cis*-3,5-dimethyl-4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid by the same procedure as employed for compound **4** as a colorless solid. Yield: 6.9 mg, 32%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.40 (6H, d, CH_3), 1.98 (2H, quintet, tetrahydropyrimidine), 3.12 (2H, m, piperazine), 3.44 (4H, br t, tetrahydropyrimidine), 3.57 (1H, dd, $CONHCH_2CH$), 3.66 (1H, dd, $CONHCH_2CH$), 3.73 (2H, m, piperazine), 3.83 (2H, m, piperazine), 3.98 (1H, m, $CONHCH_2CH$), 7.01 (2H, d, C_6H_4), 7.48 (2H, m, C_6H_5), 7.55 (1H, m, C_6H_5), 7.75 (2H, d, C_6H_4), 7.85 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{26}H_{34}N_6O_5S$ 543.2390, found 543.2380; $[\alpha]_D^{25}$ +79° (*c* 0.28, MeOH).

5.22. Preparation of compound 45

5.22.1. Ethyl 4-([1,4]diazepan-1-yl)benzoate. Ethyl 4-fluorobenzoate (1.2 g, 7.1 mmol) was added to homopiperazine (1.4 g, 14 mmol) to prepare a suspension. The suspension was stirred at 120 °C for 7.5 h and cooled to room temperature, followed by purification by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 900:100:1) to prepare the title compound (1.1 g, 62%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 (3H, t, Et), 1.89 (2H, m, homopiperazine), 2.82 (2H, m, homopiperazine), 3.03 (2H, m, homopiperazine), 3.61 (4H, m, homopiperazine), 4.31 (2H, q, Et), 6.66 (2H, d, C_6H_4), 7.89 (2H, d, C_6H_4); TSPMS *m/z* 249 $(M+H)^+$.

5.22.2. Ethyl 4-{4-(pyrimidin-2-yl)-[1,4]diazepan-1-yl}benzoate. The title compound was prepared from ethyl 4-([1,4]diazepan-1-yl)benzoate by the same procedure as employed for compound **6** as a colorless solid. Yield: 200 mg, 61%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 (3H, t, Et), 2.10 (2H, quintet, homopiperazine), 3.56 (2H, t, homopiperazine), 3.65 (2H, t, homopiperazine), 3.70 (2H, dd, homopiperazine), 3.99 (2H, dd, homopiperazine), 4.32 (2H, q, Et), 6.47 (1H, t, pyrimidine), 6.70 (2H, d, C_6H_4), 7.90 (2H, d, C_6H_4), 8.28 (2H, d, pyrimidine); APCIMS *m/z* 327 $(M+H)^+$.

5.22.3. 4-{4-(Pyrimidin-2-yl)-[1,4]diazepan-1-yl}benzoic acid. The title compound was prepared from ethyl 4-{4-(pyrimidin-2-yl)-[1,4]diazepan-1-yl}benzoate by the same procedure as employed for compound **7** as a colorless solid. Yield: 94 mg, 100%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.12 (2H, quintet, homopiperazine), 3.58 (2H, t, homopiperazine), 3.67 (2H, t, homopiperazine), 3.73 (2H, m, homopiperazine), 4.01 (2H, m, homopiperazine), 6.49 (1H, t, pyrimidine), 6.73 (2H, d, C_6H_4), 7.95 (2H, d, C_6H_4), 8.30 (2H, d, pyrimidine); TSPMS *m/z* 299 $(M+H)^+$.

5.22.4. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-yl)-[1,4]diazepan-1-yl}benzoylamino]propionate. The title compound was prepared from 4-{4-(pyrimidin-2-yl)-[1,4]diazepan-1-yl}benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 172 mg, 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 2.10 (2H, quintet, homopiperazine), 3.55 (2H, t, homopiperazine), 3.58 (1H, ddd, $CONHCH_2CH$), 3.68 (4H, m, homopiperazine), 3.86 (1H, ddd, $CONHCH_2CH$), 3.93 (1H, m, $CONHCH_2CH$), 4.01 (2H, m, homopiperazine), 6.47 (1H, t, pyrimidine), 6.71 (2H, d, C_6H_4), 7.47 (2H, m, C_6H_5), 7.54 (1H, m, C_6H_5), 7.68 (2H, d, C_6H_4), 7.86 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); APCIMS *m/z* 581 $(M+H)^+$.

5.22.5. (2*S*)-Benzenesulfonylamino-3-[4-{4-(pyrimidin-2-yl)-[1,4]diazepan-1-yl}benzoylamino]propionic acid. The title compound was prepared from *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-yl)-[1,4]diazepan-1-yl}benzoylamino]propionate by the same procedure as employed for compound **10** as a colorless solid. Yield: 210 mg, 58%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.96 (2H, quintet, homopiperazine), 3.39 (1H, dd, $CONHCH_2CH$), 3.50 (2H, m, homopiperazine), 3.56 (1H, dd, $CONHCH_2CH$), 3.60 (2H, m, homopiperazine), 3.66 (2H, m, homopiperazine), 3.91 (2H, m, homopiperazine), 3.99 (1H, dd, $CONHCH_2CH$), 6.43 (1H, t, pyrimidine), 6.67 (2H, d, C_6H_4), 7.27 (2H, m, C_6H_5), 7.32 (1H, m, C_6H_5), 7.48 (2H, d, C_6H_4), 7.70 (2H, m, C_6H_5), 8.17 (2H, d, pyrimidine); TSPMS *m/z* 525 $(M+H)^+$; $[\alpha]_D^{25}$ +37° (*c* 0.068, MeOH).

5.22.6. Compound 45. Compound **45** was prepared from (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-yl)-[1,4]-diazepan-1-yl}benzoylamino]propionic acid by the same procedure as employed for compound **4** as a pale yellow solid. Yield: 34 mg, 41%; ^{1}H NMR (400 MHz, CD_3OD) (as hydrochloride) δ: 1.73 (2H, quintet, tetrahydropyrimidine), 1.97 (2H, quintet, homopiperazine), 3.25 (4H, m, tetrahydropyrimidine), 3.45 (1H, dd, $CONHCH_2CH$), 3.49 (2H, m, homopiperazine), 3.65 (2H, m, homopiperazine), 3.70 (2H, m, homopiperazine), 3.72 (1H, dd, $CONHCH_2CH$), 3.82 (2H, m, homopiperazine), 4.18 (1H, dd, $CONHCH_2CH$), 6.80 (2H, d, C_6H_4), 7.43 (2H, m, C_6H_5), 7.50 (1H, m, C_6H_5), 7.68 (2H, d, C_6H_4), 7.82 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_5S$ 529.2233, found 529.2239; $[\alpha]_D^{25}$ +38° (*c* 0.14, MeOH).

5.23. Preparation of compound 46

5.23.1. Ethyl 4-[4-{(1*H*-benzimidazol-2-yl)methyl}piperazin-1-yl]benzoate. DMF (5.0 ml) and acetone (10 ml) were added to compound **5** (250 mg, 1.1 mmol) to prepare a solution, and 2-(chloromethyl)benzimidazole (180 mg, 1.1 mmol) and K_2CO_3 (300 mg) were added to the solution. The mixture was stirred at room temperature for 25 h and then water (200 ml) was added

thereto. After extracting with CH_2Cl_2 (100 ml) four times, the organic layer was collected. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/concd NH_4OH = 90:10:1) to prepare the title compound (274 mg, 70%) as a colorless solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.37 (3H, t, Et), 2.73 (4H, br t, piperazine), 3.37 (4H, br t, piperazine), 3.90 (2H, s, CH_2), 4.33 (2H, q, Et), 6.87 (2H, d, C_6H_4), 7.45 (2H, m, benzimidazole), 7.75 (2H, m, benzimidazole), 7.94 (2H, d, C_6H_4); TSPMS *m*/*z* 365 $(M+H)^+$.

5.23.2. 4-[4-{(1*H*-Benzimidazol-2-yl)methyl}piperazin-1-yl]benzoic acid. The title compound was prepared from ethyl 4-[4-{(1*H*-benzimidazol-2-yl)methyl}piperazin-1-yl]benzoate by the same procedure as employed for compound **7** as a colorless solid. Yield: 36 mg, 39%; ^1H NMR (400 MHz, CD_3OD) δ: 2.61 (4H, br t, piperazine), 3.29 (4H, br t, piperazine), 3.77 (2H, s, CH_2), 6.85 (2H, d, C_6H_4), 7.13 (2H, dd, benzimidazole), 7.44 (2H, dd, benzimidazole), 7.77 (2H, m, C_6H_4); TSPMS *m*/*z* 337 $(M+H)^+$.

5.23.3. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(1*H*-benzimidazol-2-yl)methyl}piperazin-1-y1]benzoyl amino]propionate. The title compound was prepared from 4-[4-{(1*H*-benzimidazol-2-yl)methyl}piperazin-1-yl]benzoic acid by the same procedure as employed for compound **9** as a colorless solid. Yield: 31 mg, 56%; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.28 (9H, s, *t*-Bu), 2.73 (4H, br t, piperazine), 3.33 (4H, br t, piperazine), 3.56 (1H, m, $CONHCH_2CH$), 3.90 (4H, m, $CONHCH_2CH$ and CH_2), 6.89 (2H, d, C_6H_4), 7.26 (2H, m, benzimidazole), 7.52 (5H, m, benzimidazole and C_6H_5), 7.72 (2H, d, C_6H_4), 7.85 (2H, m, C_6H_5); FABMS *m*/*z* 619 $(M+H)^+$.

5.23.4. Compound 46. Compound **46** was prepared from *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(1*H*-benzimidazol-2-yl)methyl}piperazin-1-y1]benzoyl amino]propionate by the same procedure as employed for compound **10** as a colorless solid. Yield: 0.64 mg, 7.0%; ^1H NMR (400 MHz, CD_3OD) δ: 2.72 (4H, br t, piperazine), 3.37 (4H, br t, piperazine), 3.53 (1H, dd, $CONHCH_2CH$), 3.65 (1H, dd, $CONHCH_2CH$), 3.82 (1H, dd, $CONHCH_2CH$), 3.88 (2H, s, CH_2), 6.96 (2H, d, C_6H_4), 7.23 (2H, dd, benzimidazole), 7.43 (2H, m, C_6H_5), 7.50 (1H, m, C_6H_5), 7.55 (2H, dd, benzimidazole), 7.68 (2H, d, C_6H_4), 7.83 (2H, m, C_6H_5); FABMS *m*/*z* 563 $(M+H)^+$.

5.24. Preparation of compound 47

5.24.1. Ethyl 4-(4-hydroxypiperidin-1-yl)benzoate. DMSO (10 ml) was added to 4-hydroxypiperidine (5.0 g, 49 mmol) to prepare a solution, and ethyl 4-fluorobenzoate (7.2 ml, 49 mmol) was added to the solution. The mixture was stirred at 120 °C for 3 days and cooled to room temperature. The reaction solution was cooled to room temperature. The reaction solution was then poured into water (200 ml) with vigorous stirring. The insolubles were collected by filtration and washed twice with water (50 ml) and then once with hexane (50 ml). The solid was dried to prepare the title compound (9.6 g, 79%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.37 (3H, t, Et), 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, Et), 6.87 (2H, d, C_6H_4), 7.91 (2H, d, C_6H_4); EIMS *m*/*z* 249 $(M)^+$.

5.24.2. Ethyl 4-(4-phthalimidopiperidin-1-yl)benzoate. Benzene (80 ml) was added to ethyl 4-(4-hydroxypiperidin-1-yl)benzoate (2.0 g, 8.1 mmol) to prepare a solution. Phthalimide (2.4 g, 16 mmol) and tributylphosphine (4.0 ml, 16 mmol) were added to the solution. The mixture was cooled to 0 °C. 1,1′-(Azodicarbonyl)-dipiperidine (4.0 g, 16 mmol) was added thereto at that temperature, and the mixture was then stirred at room temperature for 23 h. Water (300 ml) was added to stop the reaction. The mixture was extracted three times with CH_2Cl_2 (300 ml). The combined organic layer was then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/acetone = 50:1) to prepare the title compound (1.6 g, 52%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.38 (3H, t, Et), 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, Et), 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 $(M+H)^+$.

5.24.3. Ethyl 4-(4-aminopiperidin-1-yl)benzoate. MeOH (56 ml) was added to ethyl 4-(4-phthalimidopiperidin-1-yl)benzoate (850 mg, 2.2 mmol) to prepare a suspension. Hydrazine monohydrate (4.5 ml) was added to the suspension, and the mixture was stirred at room temperature for 16 h. The reaction solution was filtered through a glass filter, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) to prepare the title compound (551 mg, 100%); ^1H NMR (400 MHz, CD_3OD) δ: 1.35 (3H, t, Et), 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, Et), 6.94 (2H, d, C_6H_4), 7.84 (2H, d, C_6H_4); TSPMS *m*/*z* 249 $(M+H)^+$.

5.24.4. Ethyl 4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate. DMF (10 ml) was added to ethyl 4-(4-aminopiperidin-1-yl)benzoate (250 mg, 1.0 mmol) to prepare a solution, and 2-bromopyrimidine (240 mg, 1.5 mmol) was added to the solution. Further, *N*,*N*-diisopropylethylamine (0.10 ml) was added thereto. The mixture was heated to 125 °C, stirred for 10 h, and then cooled to room temperature. Saline (150 ml) was then added thereto, followed by extraction three times with CH_2Cl_2 (150 ml). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate = 1:1) to prepare the title compound (212 mg, 65%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.37 (3H, t, Et), 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, Et), 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 $(M)^+$.

5.24.5. 4-{4-(Pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid. THF (9.0 ml) and MeOH (3.0 ml) were added to ethyl 4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (100 mg, 0.31 mmol) to prepare a solution, and a 1 N aqueous NaOH (3.0 ml) was added to the solution. The mixture was stirred at 40 °C for 8 h and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH = 30:10:1) to prepare the title compound (75 mg, 81%); ^1H 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 $(M)^+$.

5.24.6. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate. CH_2Cl_2 (1.0 ml) and DMF (1.0 ml) were added to 4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (6.0 mg, 0.020 mmol) to prepare a solution. *N*,*N*-Diisopropylethylamine (6 μl) and benzotriazol-1-yloxy tri(dimethylamino)phosphonium hexafluorophosphate (13 mg) were added to the solution. The mixture was stirred at room temperature for 3 h. The reaction solution was added to a solution of *t*-butyl (2*S*)-*N*-benzenesulfonyl-2,3-diaminopropionate hydrochloride (8.1 mg) in CH_2Cl_2 (1.0 ml) cooled to −10 °C. Further, *N*,*N*-diisopropylethylamine (6 μl) was added thereto, followed by stirring at that temperature for 2 h. The reaction solution was concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography on silica gel ($CHCl_3$/MeOH = 10:1) to prepare the title compound (7.0 mg, 60%); ^1H 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, $CONHCH_2CH$), 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).

5.24.7. (2*S*)-Benzenesulfonylamino-3-[4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid. CH_2Cl_2 (0.30 ml) was added to the *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (7.0 mg, 0.012 mmol) to prepare a solution. Trifluoroacetic acid (0.3 ml) was added at 0 °C to the solution. The mixture was stirred at room temperature for 2 h. The reaction solution was concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography on silica gel ($CHCl_3$/MeOH/acetic acid = 10:2:1) and then purified by Sephadex LH-20 (MeOH) to prepare the title compound (6.3 mg, 100%); ^1H 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, $CONHCH_2CH$), 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).

5.24.8. Compound 47. Acetic acid (5.0 ml) and concentrated hydrochloric acid (0.50 ml) were added to (2*S*)-benzenesulfonylamino-3-[4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (3.6 mg, 0.0069 mmol) to prepare a solution. 10% Pd/C (1.8 mg) was added to the solution, and the mixture was vigorously shaken under a hydrogen pressure of 3 atm at room temperature for 2.5 h. The insolubles were filtered and washed twice with water and twice with MeOH. The filtrate was combined with the washings, followed by concentration under reduced pressure. The residue was subjected to azeotropic distillation twice each with toluene, followed by purification by preparative thin-layer chromatography on silica gel (CH_2Cl_2/EtOH/H_2O/concd NH_4OH = 8:8:1:1) and then by Sephadex LH-20 (MeOH) to prepare compound **47** (3.2 mg, 88%); ^1H NMR (400 MHz, CD_3OD) δ: 1.49 (2H, br q, J = 10.8, piperidine), 1.85 (4H, m, piperidine and tetrahydropyrimidine), 2.84 (2H, ddd, J = 2.6, 10.8, 13.5, piperidine), 3.26 (4H, t, J = 5.8, tetrahydropyrimidine), 3.45 (2H, m, $CONHCH_2CH$ and piperidine), 3.53 (1H, dd, J = 5.0, 13.4, $CONHCH_2CH$), 3.63 (1H, dd, J = 5.0, 8.0, $CONHCH_2CH$), 3.73 (2H, br d, J = 13.5, piperidine), 6.85 (2H, d, J = 9.0, C_6H_4), 7.37 (2H, m, C_6H_5), 7.44 (1H, m, C_6H_5), 7.59 (2H, d, J = 9.0, C_6H_4), 7.75 (2H, m, C_6H_5); ^{13}C NMR (DMSO-d_6) δ: 19.8, 31.2, 31.9, 37.8, 42.4, 46.6, 47.1, 55.2, 114.1, 123.9, 126.6, 126.8, 128.1, 129.0, 129.2, 132.3, 140.6, 152.1, 152.8, 165.0, 173.1; FABMS *m*/*z* 529 $(M+H)^+$; $[\alpha]_D^{20}$ +69° (*c* 0.16, MeOH).

5.25. Preparation of compound 48

5.25.1. Ethyl 4-[4-[{*N*-methyl-*N*-(pyrimidin-2-yl)}amino]piperidin-1-yl]benzoate. DMF (3.6 ml) was added to ethyl 4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (179 mg, 0.55 mmol) to prepare a solution. Methyl iodide (156 mg) was added to the solution. 60% sodium hydride in oil (43.9 mg, 1.1 mmol) was added thereto, and a reaction was allowed to proceed at 45 °C for 11 h. The reaction solution was extracted with ethyl acetate (200 ml). The extract was washed with distilled water, an aqueous $NaHCO_3$ solution, and saline, and then dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and dried to prepare the title compound (186 mg, 99%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.37 (3H, t, Et), 1.80 (2H, br d, piperidine), 1.90 (2H, dq, piperidine), 3.01 (3H, s, Me), 3.04 (2H, br dt, piperidine), 3.99 (2H, br d, piperidine), 4.33 (2H, q, Et), 4.90 (1H, dddd, piperidine), 6.48 (1H, t, pyrimidine), 6.90 (2H, d, C_6H_4), 7.93 (2H, d, C_6H_4), 8.32 (2H, d, pyrimidine); TSPMS *m*/*z* 341 $(M+H)^+$.

5.25.2. 4-[4-[{*N*-Methyl-*N*-(pyrimidin-2-yl)}amino]piperidin-1-yl]benzoic acid. The title compound was prepared from ethyl 4-[4-[{*N*-methyl-*N*-(pyrimidin-2-yl)}amino]piperidin-1-yl]benzoate by the same procedure as employed for compound **7**. Yield: 102 mg, 55%; ^1H NMR (400 MHz, 67% CD_3OD/$CDCl_3$) δ: 1.80 (2H, br d, piperidine), 1.94 (2H, dq, piperidine), 3.01 (3H, s, Me), 3.03 (2H, br t, piperidine), 4.06 (2H, br d, piperidine),

4.86 (1H, dddd, piperidine), 6.57 (1H, t, pyrimidine), 6.96 (2H, d, C_6H_4), 7.91 (2H, d, C_6H_4), 8.32 (2H, d, pyrimidine); TSPMS *m/z* 313 $(M+H)^+$.

5.25.3. *t*-Butyl (2*S*)-benzenesulfonylamino-3-[4-[4-[{*N*-methyl-*N*-(pyrimidin-2-yl)}amino]piperidin-1-yl] benzoylamino]-propionate. The title compound was prepared from 4-[4-[{*N*-methyl-*N*-(pyrimidin-2-yl)}amino]piperidin-1-yl]benzoic acid by the same procedure as employed for compound **9**. Yield: 303 mg, 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 1.80 (2H, br d, piperidine), 1.92 (2H, dq, piperidine), 3.02 (3H, s, Me), 3.02 (2H, br t, piperidine), 3.58 (1H, ddd, $CONHCH_2CH$), 3.85-4.00 (4H, m, $CONHCH_2CH$, $CONHCH_2CH$ and piperidine), 4.89 (1H, dddd, piperidine), 6.48 (1H, t, pyrimidine), 6.92 (2H, d, C_6H_4), 7.49 (2H, br t, C_6H_5), 7.57 (1H, br t, C_6H_5), 7.71 (2H, d, C_6H_4), 7.86 (2H, m, C_6H_5), 8.32 (2H, d, pyrimidine); TSPMS *m/z* 595 $(M+H)^+$; $[\alpha]_D^{25}$ +55° (*c* 2.0, CH_2Cl_2).

5.25.4. (2*S*)-Benzenesulfonylamino-3-[4-[4-[{*N*-methyl-*N*-(pyrimidin-2-yl)}amino]piperidin-1-yl]benzoyl amino]propionic acid. The title compound was prepared from *t*-butyl (2*S*)-benzenesulfonylamino-3-[4-[4-[{*N*-methyl-*N*-(pyrimidin-2-yl)}amino]piperidin-1-yl]benzoylamino]propionate by the same procedure as employed for compound **10**. Yield: 262 mg, 99%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.77 (2H, br d, piperidine), 1.95 (2H, dq, piperidine), 2.96 (2H, br t, piperidine), 3.00 (3H, s, Me), 3.55 (1H, dd, $CONHCH_2CH$), 3.66 (1H, dd, $CONHCH_2CH$), 3.81 (1H, dd, $CONHCH_2CH$), 4.03 (2H, br d, piperidine), 6.57 (1H, t, pyrimidine), 7.00 (2H, d, C_6H_4), 7.45 (2H, m, C_6H_5), 7.52 (1H, m, C_6H_5), 7.69 (2H, d, C_6H_4), 7.85 (2H, m, C_6H_5), 8.32 (2H, d, pyrimidine); TSPMS *m/z* 539 $(M+H)^+$; $[\alpha]_D^{25}$ +78° (*c* 0.50, MeOH/$CHCl_3$ = 1:1).

5.25.5. Compound 48. Compound **48** was prepared from (2*S*)-benzenesulfonylamino-3-[4-[4-[{*N*-methyl-*N*-(pyrimidin-2-yl)}amino]piperidin-1-yl]benzoyl amino]propionic acid by the same procedure as employed for compound **4**. Yield: 29 mg, 78%; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.74 (2H, br d, piperidine), 1.86 (2H, dq, piperidine), 1.93 (2H, quintet, tetrahydropyrimidine), 2.78 (3H, s, Me), 2.86 (2H, br t, piperidine), 3.38 (4H, t, tetrahydropyrimidine), 3.57 (1H, dd, $CONHCH_2CH$), 3.65 (1H, dd, $CONHCH_2CH$), 3.73 (1H, dd, $CONHCH_2CH$), 3.92 (2H, br d, piperidine), 6.92 (2H, d, C_6H_4), 7.47 (2H, m, C_6H_5), 7.53 (1H, m, C_6H_5), 7.70 (2H, d, C_6H_4), 7.86 (2H, m, C_6H_5); FABMS *m/z* 543 $(M+H)^+$; $[\alpha]_D^{25}$ +83° (*c* 1.0, MeOH).

5.26. 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). $\alpha_v\beta_3$[14] was purified from human placenta, using RGDSPK-Sepharose CL-4B chromatography, followed by mono Q chromatography (Pharmacia). $\alpha_{IIb}\beta_3$[14] 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.[15] EIA plates (Nunc) were coated with $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ and blocked with bovine serum albumin. In each reaction, the reaction mixture (20 mM Tris–HCl, 150 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4, 100 μl) including vitronectin (Calbiochem) or fibrinogen, added to the receptor-coated plate, was incubated for 4 h at 25°C. Thereafter the ligand binding was measured using anti-vitronectin rabbit antibody (Calbiochem) and peroxidase-conjugated anti-rabbit IgG antibody (Capell) for $\alpha_v\beta_3$, or peroxidase-conjugated anti-fibrinogen antibody (Capell) for $\alpha_{IIb}\beta_3$, and 2, 2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) as the substrate of peroxidase. The IC_{50} values were determined from measurement of absorbance at 415 nm.

5.27. Adhesion of human aorta smooth muscle cells to vitronectin

The adhesion of human aorta smooth muscle cells to vitronectin was measured as described before.[18] Briefly, EIA plates (Nunc) were coated with human vitronectin (Calbiochem) and blocked with bovine serum albumin. The cell suspension of human aorta smooth muscle cells (50,000 cells/100 μl, Clonetics) in Dulbecco's modified Eagle's basal medium containing 0.1% bovine serum albumin was added to the vitronectin-coated plates and incubated for 1.5 h at 37 °C in the presence or absence of the test compounds. The adherent cells were stained with toluidine blue and calculated by the measuring of absorbance at 405 nm after the cytolysis by SDS. The IC_{50} values were determined graphically from two or more independent experiments.

5.28. Platelet aggregation assay

Platelet aggregation was determined according to the previous method.[15] Human platelet-rich plasma obtained from healthy volunteers was prepared and the aggregation was induced with 5 μM ADP. The IC_{50} values were determined from two independent experiments.

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