Structure−Activity Relationship of Small-Sized HIV Protease Inhibitors Containing Allophenylnorstatine

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We designed and synthesized a new class of peptidomimetic human immunodeficiency virus (HIV) protease inhibitors containing a unique unnatural amino acid, allophenylnorstatine [Apns; (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid], with a hydroxymethylcarbonyl (HMC) isostere as the active moiety. A systematic evaluation of structure−activity relationships for HIV protease inhibition, anti-HIV activities, and pharmacokinetic profiles has led to the delineation of a set of structural charateristics that appear to afford an orally available HIV protease inhibitor. Optimum structures, exemplified by **21f** (JE-2147), incorporated 3-hydroxy-2-methylbenzoyl groups as the P2 ligand, (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carbonyl (Dmt) residue at the P1′ site, and 2-methylbenzylcarboxamide group as the P2′ ligand. The present study demonstrated that JE-2147 has potent antiviral activities in vitro and exhibits good oral bioavailability and plasma pharmacokinetic profiles in two species of laboratory animals.

Introduction

The alarming spread of human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), has initiated an urgent pursuit to comprehend and control this disease. Advances in molecular, viral, and cell biology have defined numerous targets for potential drug intervention. The virally encoded homodimeric aspartyl protease, which is responsible for processing the *gag* and *gag/pol* gene products that allow for the organization of core structural proteins and release of viral enzymes, is one such target.[1] Inhibition of this enzyme prevents the maturation and replication of the virus in cell culture. Inhibitors of HIV protease are presently being used in therapy for the treatment of AIDS.[2] The HIV-1 protease can recognize Phe-Pro and Tyr-Pro sequences as the retrovirus-specific cleavage site, but mammalian aspartic proteases such as renin, pepsin, and cathepsin D do not have such specificity. These features provided a basis for the rational design of selective HIV protease-targeted drugs for the treatment of AIDS and related diseases. Previously, we reported a series of peptide mimetic HIV protease inhibitors containing allophenylnorstatine [Apns, (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] with a hydroxymethylcarboxamide (HMC) isostere based on the transition-state mimic concept.[3,4] The compounds containing a thiazolidine ring, a bioisostere of proline, at the P1′ portion showed highly potent HIV protease inhibitory activity.[5,6] The compounds containing a 5-isoquinolinyloxyacetyl group at the P3 site, represented by KNI-227 (**1**) and KNI-272 (**2**), (Figure 1), showed highly potent anti-HIV activity, and KNI-272 was well-absorbed from the digestive tract in rats and dogs. Although KNI-272 showed a good oral bioavailability in a human clinical trial, the plasma half-life was very short ($t_{1/2\beta}$ = 23 min upon intravenous administration),[7] and tid or qid regimen was needed to maintain a plasma concentration for over 90% inhibition of HIV replication.[8]

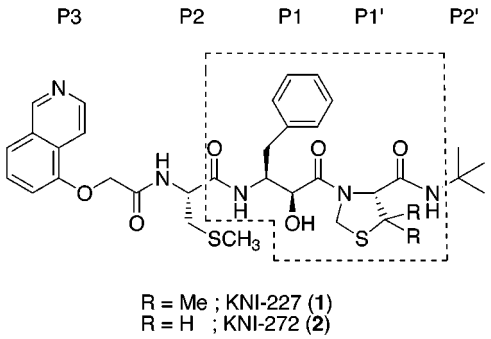

Figure 1. Structures of KNI-227 (**1**) and KNI-272 (**2**).

In the present study, we describe our continuing effort to generate novel small-sized HIV protease inhibitors bearing desirable antiviral activity and pharmacokinetic profiles, which ultimately resulted in JE-2147, a potent and orally bioavailable HIV protease inhibitor.

Design Rationale

Information on the interaction between KNI-272 and HIV-1 protease based on studies of X-ray crystallography of the complex,[9] NMR in the solution state,[10] and molecular modeling[11] gave us the basic structure to design novel small-sized HIV protease inhibitors. The hydroxymethylcarbonyl group of Apns (P1) interacts with the aspartic acid carbonyl groups of the HIV-1 protease active site in essentially the same hydrogen-bonding mode as occurs in the transition state and contributes to the high activity of KNI-272.[10] The P2 and P1′ carbonyl moieties in KNI-272 are in position to maintain a critical hydrogen bond to a water molecule, which hydrogen bonds to the backbone of both flaps

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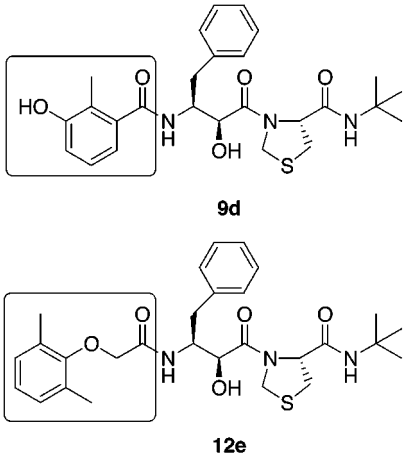


Figure 2. Structures of small-sized lead compounds.

(Ile50 and Ile50′). Moreover, the amide nitrogen of *tert*-butyl (P2′) binds to a water molecule, which forms bridging hydrogen bonds between the amide nitrogen atom of the P2′ group and the backbone nitrogen of Asp29. This causes the 10-fold preference in inhibitory potency of the amide linkage over the ester linkage of KNI-272 analogues. Another feature that may relate to the high potency of KNI-272 is that the conformationally constrained P1−P1′ linkage binds in a low-energy trans conformation. In contrast to most peptidomimetic inhibitors of HIV protease, which exhibit a high degree of conformational flexibility, KNI-272 appears to be structurally preorganized to bind in an energetically preferred conformation. Therefore the basic structure (surrounded by the dashed line in Figure 1) was selected, and P2 and P2′ ligands were mainly tested in the course of our structure−activity relationship (SAR) study on HIV-1 protease inhibition.

Recently, a variety of ligands optimized for binding to the S2 subsite of HIV protease have been reported for hydroxyethylamine inhibitors. In particular, the 3-hydroxy-2-methylbenzoyl group[12−16] and 2,6-dimethylphenoxyacetyl group[17,18] provide attractive P2 ligands due to their lack of stereocenters and ease of synthesis. Therefore, we hypothesized that a small-sized inhibitor incorporating these groups into the backbone of KNI-272 series molecules might show improved plasma stability while maintaing its highly potent antiviral activity. Two types of small-sized lead compounds (Figure 2, **9d**, **12e**) were designed, and a SAR study of P2 and P1′ sites was performed. On the other hand, the retroviral proteases in general, and HIV protease in particular, have been shown to exist in their active forms as C_2-symmetric homodimers with each monomer contributing a catalytic triad to a single active site. The high degree of symmetry of the HIV protease structure offers a unique opportunity for the design of C_2-symmetric inhibitors that match the characteristics of the enzyme structure. Many inhibitors based on this character were synthesized, and some of them showed excellent antiviral properties.[19−21] This character prompted us to introduce an aryl group as the P2′ ligand. The C_2 operation along the axis in the Apns-Thz site of compound **12e** generated the benzylcarboxamide group as a novel P2′ ligand (Figure 3). The amide nitrogen of P2′ was expected to interact with the HIV-1 protease in a similar manner as the amide nitrogen of the *tert*-butyl (P2′) of KNI-272. Thus, in addition to the benzoyl (P2)-type compounds, another type of lead compound (**21a**) was obtained.

Chemistry

Boc-Apns-OH[22,23] and 2-alkyl-3-hydroxybenzoic acids[24] were prepared according to the methods described previously.

Scheme 1 illustrates the procedures to synthesize dipeptide *tert*-butylamide derivatives **6a,b**. Boc-protected 1,3-thiazolidine-4-carboxylic acid derivatives **4a,b** were prepared from the corresponding cysteine analogues **3a,b** by cyclization with formaldehyde, followed by *tert*-butoxycarbonylation with Boc_2O in a one-pot reaction. The amide bond formation here was prepared by use of *N*,*N*′-dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt) or diphenylphosphoryl chloride (DPP-Cl) as a condensation reagent. The mixed anhydride prepared with DPP-Cl was effective for preparation of Boc-protected 5,5-dimethyl-1,3-thiazolidine-4-carboxamides.

The inhibitors in Table 1 (**8a,b,e** and **9c,d,f−i**) were obtained as shown in Scheme 2. The 3-hydroxyl group

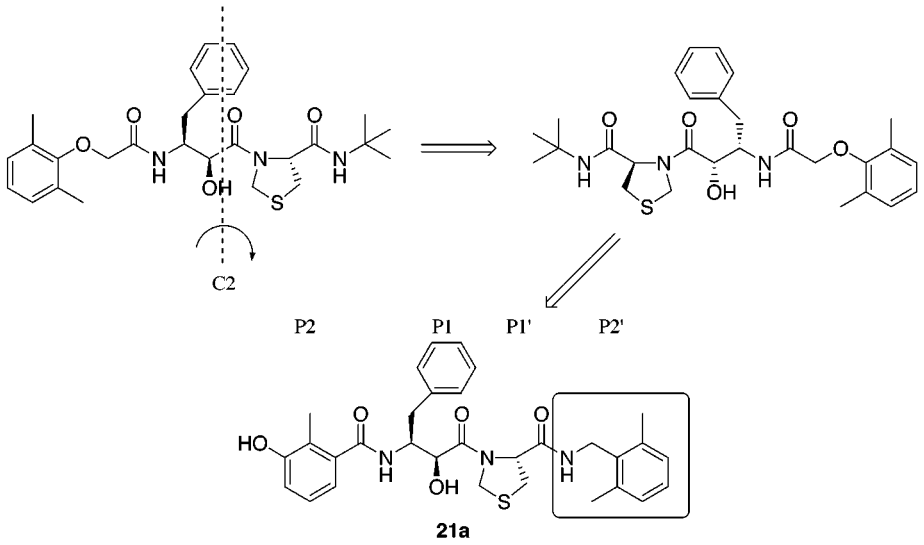
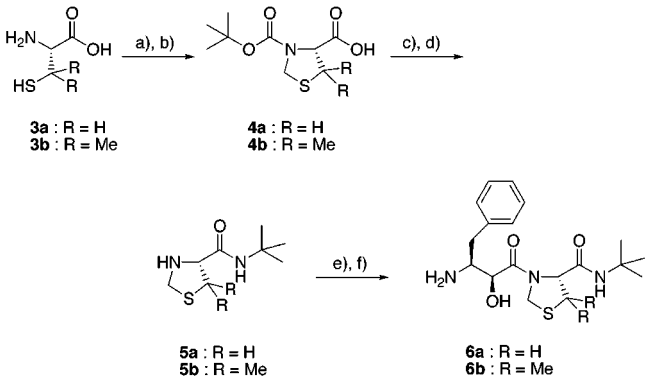


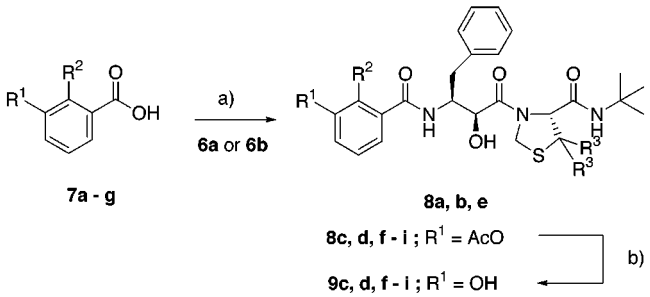
Figure 3. Design of symmetric type compounds.

Scheme 1[a]



[a] Conditions: (a) $HCHO_{aq}$; (b) Boc_2O, $NaOH_{aq}$, THF; (c) DCC-HOBt, *tert*-butylamine, CH_2Cl_2; or DPP-Cl, triethylamine, *tert*-butylamine, ethyl acetate; (d) methanesulfonic acid/CH_2Cl_2; (e) Boc-Apns-OH, EDC-HOBt, DMF; (f) methanesulfonic acid/CH_2Cl_2.

Scheme 2[a]



7 and 8	R^1	R^2	R^3
a	H	H	H
b	H	Me	H
c	AcO	H	H
d	AcO	Me	H
e	NH_2	Me	H
f	AcO	Et	H
g	AcO	Pr	H
h	AcO	Me	Me
i	AcO	Et	Me

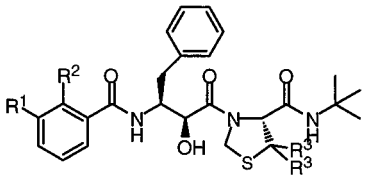
[a] Conditions: (a) EDC, HOBt, DMF; or DPP-Cl, triethylamine, ethyl acetate; (b) $NaOH_{aq}$, methanol.

on the benzoic acids was protected with an acetyl group during the course of the coupling step and were saponified in the final stage.

Scheme 3 illustrates the procedures to synthesize the aryloxyacetyl-type compounds shown in Table 2 (**12a**−**i**). All phenoxyacetic acids **11a**−**i** were obtained by the reaction of phenols and methyl chloroacetate in the presence of K_2CO_3, followed by saponification. The coupling step for these compounds was performed by the *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide (EDC)−HOBt method.

The methods for synthesis of the benzylamide-type compounds (Table 3) are shown in Schemes 4 and 5. All commercially available benzylamines were purchased. 2,6-Dimethylbenzylamine (**16a**) was synthesized according to Scheme 4. 2,6-Dimethylbenzoic acid (**13**) was converted to the corresponding benzyl alcohol **14** by esterification with methyl iodide, followed by reduction with $LiAlH_4$. 2,6-Dimethylbenzyl chloride, which

Table 1. HIV-1 Protease Inhibitory Activity of Benzoyl-Type Compounds



	structure			HIV-1 protease inhibition (%)		
compd	R^1	R^2	R^3	at 5 μM	at 50 nM	K_i (nM)
8a	H	H	H	52	nt[a]	nt
8b	H	Me	H	75	nt	nt
9c	OH	H	H	76	nt	nt
9d	OH	Me	H	97	36	24.9
8e	NH_2	Me	H	81	nt	nt
9f	OH	Et	H	98	46	22.2
9g	OH	Pr	H	92	17	nt
9h (JE-533)	OH	Me	Me	99	88	5.14
9i	OH	Et	Me	99	84	2.24

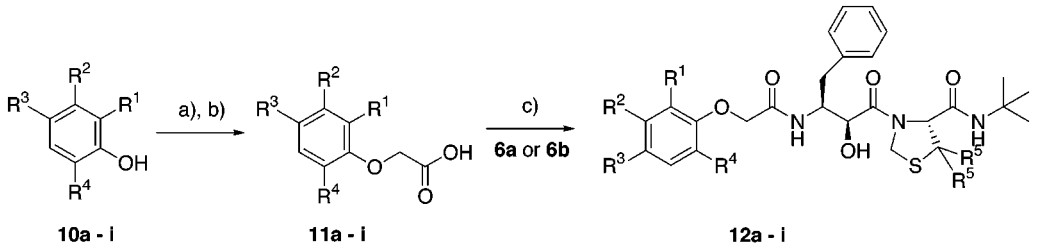
[a] nt, not tested.

was obtained by chlorination of the corresponding benzyl alcohol with trimethylsilyl chloride (TMS-Cl) in the presence of dimethyl sulfoxide (DMSO),[25] was converted to 2,6-dimethylbenzylamine (**16a**) by the use of a Gabriel-type reaction. Reduction of 2,6-dimethylbenzamide with $LiAlH_4$ in tetrahydrofuran (THF) under reflux condition gave the corresponding benzyl alcohol, not benzylamine. The synthesis of inhibitors shown in Table 3 (**21a**−**i**) was conducted by the same method used for the inhibitors in Table 1 (Scheme 5).

Results and Discussion

SAR Study on HIV-1 Protease Inhibition. The compounds synthesized in this study were first tested for HIV-1 protease inhibitory activity. HIV-1 protease activity was determined by an HPLC method using recombinant HIV-1 protease (NY-5) and synthetic peptide H-Ser-Gln-Asn-Tyr-Pro-Ile-Val-OH as a substrate. Table 1 shows the results of the SAR study on HIV-1 protease inhibition by the benzoyl-type compounds. The compound **9d** bearing both *o*-methyl group and *m*-hydroxyl group of the phenyl ring at the P2 site exhibited high HIV protease inhibitory activity (K_i = 24.9 nM). The compounds having only one of these groups, either the methyl group (**8b**) or the hydroxyl group (**9c**), showed weak activity. These results are supported by the observation of Kalish et al.[12] that the methyl group provides additional binding over the corresponding unsubstituted analogue through increased hydrophobic interactions with the S2 pocket and that the methyl group induces repulsion between the aromatic ring and the amide carbonyl, such that the hydroxyl group at the 3 position is oriented in a favorable position for hydrogen-bonding interaction with Asp30. Replacement of the *o*-methyl group with an ethyl group (**9f**) resulted in a slightly increased enzyme inhibitory activity, but the replacement with the bulkier propyl group (**9g**) did not increase the activity. An amino group at the R^1 caused a decrease in the enzyme inhibitory activity (**8e**). The amino group, a weaker hydrogen bond donor than the hydroxyl group, is considered to not make a good hydrogen bond with

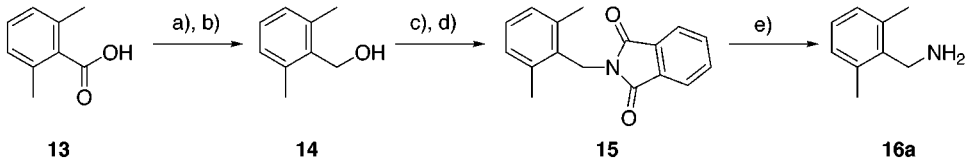
Scheme 3[a]



10 - 12	R^1	R^2	R^3	R^4	R^5
a	H	H	H	H	H
b	Me	H	H	H	H
c	H	Me	H	H	H
d	H	H	Me	H	H
e	Me	H	H	Me	H
f	Me	H	H	Et	H
g	Me	H	H	Pr	H
h	Et	H	H	Et	H
i	Et	H	H	Et	Me

[a] Conditions: (a) Cl-CH_2COOMe, K_2CO_3, DMF; (b) $NaOH_{aq}$, methanol; (c) EDC, HOBt, DMF.

Scheme 4[a]

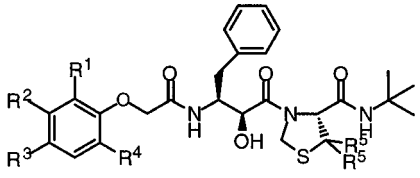


[a] Conditions: (a) methyl iodide, K_2CO_3, DMF; (b) $LiAlH_4$, THF; (c) trimethylsilyl chloride, DMSO; (d) potassium phthalimide, DMF; (e) NH_2NH_2, ethanol, and then concd HCl.

Asp30. We previously found that the methyl substitution at the 5 position of the thiazolidine ring at the P1′ portion of the tripeptide inhibitors remarkably enhanced the protease inhibitory activity (KNI-272 (**2**) vs KNI-227 (**1**), K_i values were 0.74 and 0.088 nM, respectively). These results prompted us to introduce methyl groups at the thiazolidine ring of **9d** or **9f**. The resulting compounds, **9h** (JE-533) and **9i**, containing 5,5-dimethyl-1,3-thiazolidine-4-carbonyl (Dmt) at the P1′ position, showed more potent HIV protease inhibitory activity, and the K_i values were 5.14 and 2.24 nM, respectively (Table 1).

Table 2 presents the results of the SAR study on phenoxyacetyl-type compounds. At first, we examined the effect of a methyl group introduced at various positions of the phenoxyacetyl group of compound **12a**. The substitution by the methyl group at the ortho position (R^1) enhanced the HIV protease inhibitory activity (**12b**), but neither meta nor para substitution increase the inhibitory activity (**12c**,**d**). These data indicated that the *o*-methyl group is essential for the increase in binding affinity for the enzyme and that there was no hydrophobic space in the meta position. Additionally, we found that *o*-methyl substitution at R^4 generated a potent HIV protease inhibitor having a 2,6-dimethylphenoxyacetyl group (**12e**). Compounds **12f,g** having a bulkier group (ethyl or propyl) at R^4 showed slightly decreased protease inhibitory activity. Compound **12h** with ethyl groups at both R^1 and R^4 showed little inhibitory activity. Thus the presence of a relatively small hydrophobic pocket at both ortho positions and a 2,6-dimethylphenoxyacetyl group[17] provided the best ligand of our HMC-type compounds. In the case of the compounds of this type, the introduction of the Dmt residue at the P1′ enhanced the enzyme inhibitory activity, and the K_i value was 1.40 nM (**12i**).

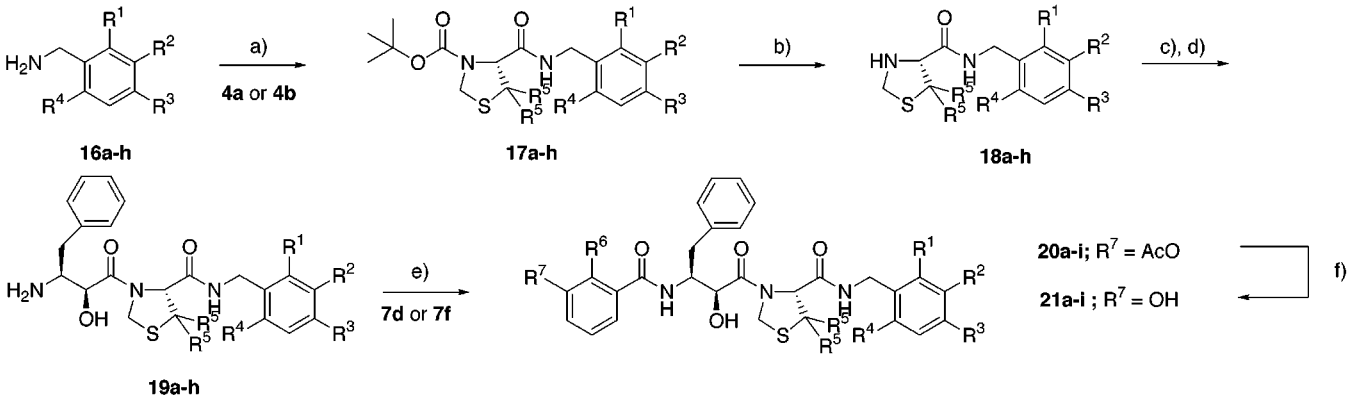
Table 2. HIV-1 Protease Inhibitory Activity of Phenoxyacetyl-Type Compounds



	structure					HIV-1 protease inhibition (%)		
compd	R^1	R^2	R^3	R^4	R^5	at 5 μM	at 50 nM	K_i (nM)
12a	H	H	H	H	H	48	nt[a]	nt
12b	Me	H	H	H	H	82	nt	nt
12c	H	Me	H	H	H	38	nt	nt
12d	H	H	Me	H	H	44	nt	nt
12e	Me	H	H	Me	H	95	56	21.7
12f	Me	H	H	Et	H	94	32	nt
12g	Me	H	H	Pr	H	97	35	nt
12h	Et	H	H	Et	H	62	nt	nt
12i (JE-1482)	Me	H	H	Me	Me	97	96	1.40

[a] nt, not tested.

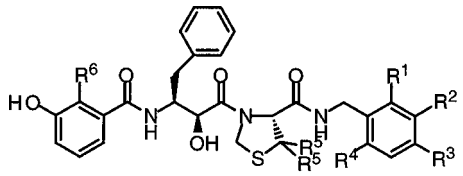
Scheme 5[a]



16 - 21	R^1	R^2	R^3	R^4	R^5	R^6
a	Me	H	H	Me	H	Me
b	H	H	H	H	H	Me
c	Me	H	H	H	H	Me
d	H	Me	H	H	H	Me
e	H	H	Me	H	H	Me
f	Me	H	H	H	Me	Me
g	Cl	H	H	H	Me	Me
h	CF_3	H	H	H	Me	Me
i	Me	H	H	H	Me	Et

[a] Conditions: (a) EDC, HOBt, DMF; or DPP-Cl, triethylamine, ethyl acetate; (b) methanesulfonic acid/CH_2Cl_2; or HCl/dioxane; (c) Boc-Apns-OH, EDC, HOBt, DMF; (d) methanesulfonic acid/CH_2Cl_2; or HCl/dioxane; (e) DPP-Cl, triethylamine, ethyl acetate; (f) $NaOH_{aq}$, methanol.

Table 3. HIV-1 Protease Inhibitory Activity of Benzylamide-Type Compounds



	structure						HIV-1 protease inhibition (%)	
compd	R^1	R^2	R^3	R^4	R^5	R^6	at 50 nM	K_i (nM)
21a	H	H	H	H	H	Me	43	59
21c	Me	H	H	H	H	Me	70	8.9
21d	H	Me	H	H	H	Me	18	nt[a]
21e	H	H	Me	H	H	Me	13	nt
21a	Me	H	H	Me	H	Me	75	4.8
21f (JE-2147)	Me	H	H	H	Me	Me	96	0.33
21g	Cl	H	H	H	Me	Me	95	0.29
21h	CF_3	H	H	H	Me	Me	88	nt
21i (JE-1520)	Me	H	H	H	Me	Et	97	0.19

[a] nt, not tested.

Table 3 shows the results of our SAR study on the benzylamide (P2′)-type compounds. Although the compounds bearing the 2,6-dimethylphenoxyacetyl group exerted a potent HIV protease inhibitory activity (Table 2), this activity was not translated into an anti-HIV activity, as described later (Table 4, **12i**). Therefore benzyl groups were introduced into the benzoyl-type compounds. Compound **21b**, bearing an unsubstituted benzyl group at the P2′ site, showed HIV-1 protease inhibitory activity comparable to that of the *tert*-butyl compound (**9d**). Substitution by an *o*-methyl group at the phenyl ring provided a compound more potent than the corresponding unsubstituted analogue **21c** ($K_i = 8.9$ nM). On the other hand, the *m*- or *p*-methyl substituent at the phenyl ring unexpectedly resulted in decreased inhibitory activity (**21d,e**). Furthermore, compound **21a** having an additional *o*-methyl substituent, i.e., the 2,6-dimethylbenzyl group, showed only slightly improved potency. These results were almost the same as in the case of the P2 phenoxyacetyl group. However, the increase in potency of benzylamide (P2′)-type compounds upon introduction of Dmt at the P1′ site (27-fold increase in comparison with **21c**,**f**, in Table 3) was easily unexpected base on the results of SAR of *tert*-

Table 4. Inhibitory Activity against HIV-1 Protease, Anti-HIV-1 IIIB Activity, and Cellular Toxicity of HIV Protease Inhibitors

	HIV-1 protease inhibition	HIV-1 IIIB	
compd	K_i (nM)	IC_{50} (nM)[a]	TC_{50} (μM)[b]
9d	24.9	>200	>20
9h	5.14	19	>20
9i	2.24	92	>20
12e	21.7	>200	>20
12i	1.40	>200	>20
21c	8.91	>200	>20
21f (JE-2147)	0.33	7	>20
21g	0.29	34	>20
21i (JE-1520)	0.16	11	>20
1 (KNI-227)	0.088	6	>20
2 (KNI-272)	0.74	34	>20

[a] Antiviral activities were determined based on the HIV-1 IIIB-induced cytopathic effects evaluated by the use of the tetrazolium reagent as described in the Experimental Section. Each value represents the mean of duplicate determinations. [b] The value shown is the 50% toxic concentration of the test compound.

Table 5. Pharmacokinetic Profiles of HIV Protease Inhibitors after Intravenous or Intraduodenal Administration in Rats[a]

	iv injection	id administration		
compd	$t_{1/2\beta}$ (min)	C_{max} (μM)	T_{max} (min)	F (%)
9d	59	2.91 ± 0.21	30	33.9 ± 3.4
9h	83	2.60 ± 0.85	30	39.4 ± 16.1
9f	74	1.73 ± 0.04	30	36.2 ± 1.2
9i	84	1.16 ± 0.18	30	37.0 ± 2.8
21c	41	0.77 ± 0.07	30	32.3 ± 10.0
21f (JE-2147)	93	0.70 ± 0.20	60	41.6 ± 10.7
21i (JE-1520)	126	0.23 ± 0.09	10	10.0 ± 1.0

[a] The values represent the mean ± standard error of the mean for three rats. F (%) was determined by comparing the mean areas under the curves (AUC) after intravenous and intraduodenal doses. $t_{1/2\beta}$, plasma half-life; C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; F (%), percent bioavailability.

butylamide (P2′)-type compounds (5-fold increase in potency in comparison with **9d**,**h**, in Table 1). Introduction of a Dmt residue at the P1′ site resulted in the highly potent protease inhibitor **21f** (JE-2147), which had $K_i = 0.33$ nM. The two methyl substituents of the thiazolidine ring presumably oriented the benzyl group to a more favorable position for interacting with the protease. Although replacement of the methyl group with chlorine (**21g**) had no effect on the activity, replacement with the bulkier CF_3 group (**21h**) decreased the activity.

Antiviral Activity. Antiviral activity for selected analogues in Tables 1−3 was determined in cell culture against HIV-1 strain IIIB in CEM-SS cells (Table 4). The compounds containing a Dmt residue at the P1′ site, except for **12i**, showed potent antiviral activity with IC_{50} values below 100 nM, and their 50% toxic concentrations were more than 20 μM. Especially, benzylamide-type compounds **21f** (JE-2147) and **21i** (JE-1520), which had low K_i values, showed a highly potent activity equal to that of the tripeptide compounds KNI-227 (**1**) and KNI-272 (**2**). Although the compounds bearing the 2,6-dimethylphenoxyacetyl group (**12e**,**i**) showed potent HIV-1 protease inhibitory activity equal to that of the benzoyl-type compounds, this action did not translate into anti-HIV activity in CEM-SS cells. The discrepancy between protease inhibitory activity and antiviral effect has not yet been clarified.

Pharmacokinetics. A representative set of compounds (**9d**−**21i**) was examined for pharmacokinetics in rats, and the results are summarized in Table 5. After intravenous (iv) administration (10 mg/kg), the elimination half-life of these compounds ranged from 41 to 126 min, which was more than 3 times longer than that of KNI-272. The plasma levels of these compounds, except compound **21i**, reached 0.70−2.91 μM, and the bioavailability was over 30% when intraduodenally (id) administered to rats as a 50% poly(ethylene glycol) (PEG) solution (10 mg/kg). The 2-methyl-3-hydroxybenzoyl-type compounds maintained higher maximum plasma concentration (C_{max}) than the 2-ethyl-3-hydroxybenzyl-type compounds (**9d** vs **9f**, **9h** vs **9i**, and **21f** vs **21i**). The *tert*-butylcarboxamide-type compounds were absorbed more quickly than the benzylcarboxamide ones. The slow absorption of the benzylcarboxamide-type compound (e.g., **21f**) contributed to the maintenance of the plasma level for a long period after id administration. Unfortunately, compound **21i**, which had excellent antiviral potency and a long plasma half-life, did not show an acceptable plasma concentration by id administration of this formulation. In the pharmacokinetic study in dogs (Table 6 and Figure 4), the experiment confirmed that **21f** (JE-2147) had a favorable pharmacokinetic profile. JE-2147 showed an elimination half-life with 94 min after iv administration, and the oral bioavailability was estimated to be 33% and 37% in the nonfasting and fasting conditions, respectively. The maximum plasma concentration achieved was 4.02−5.30 μM when JE-2147 was orally administered at a dose of 25 mg/kg to dogs (Table 6). In addition, a single oral dose (25 mg/kg) of JE-2147 exhibited plasma levels exceeding the in vitro antiviral IC_{95} (52 nM) for more than 12 h in dogs (Figure 4). Additional antiviral testing demonstrated that JE-2147 was effective against the replication of HIV-1 IIIB in various cells (T cell, B cell, macrophage, and PBMC) with IC_{50} values ranging from 31 to 160 nM and also had antiviral activity against simian immunodefidiency virus and HIV-2 as effective as that of other HIV protease inhibitors and reverse transcriptase inhibitors.[26] Moreover, the resistance

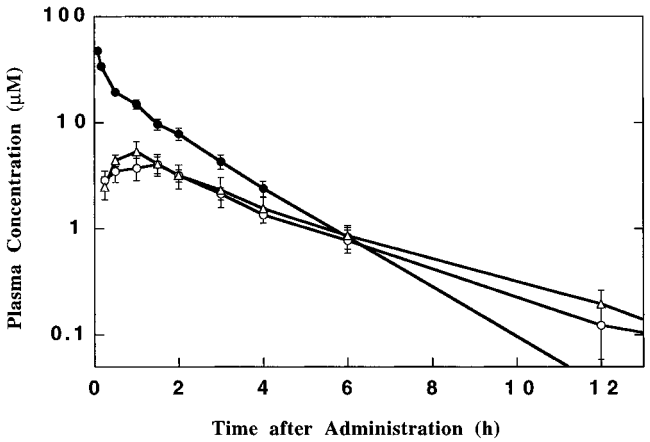


Figure 4. Plasma concentrations of JE-2147 after dosing at 25 mg/kg by intravenous or oral administration to dogs. JE-2147 was formulated in 50% PEG. The plasma concentration was measured by the HPLC method as described in the Experimental Section. Each point represents the mean ± SE of six dogs: ●, iv injection; ○, po administration (fed); △, po administration (fasted 16 h).

Table 6. Oral Pharmacokinetics of JE-2147 in Dogs under Various Feeding Conditions[a]

feeding condition	route	dose (mg/kg)	AUC (μM·min)	F (%)	C_{max} (μM)	T_{max} (min)	$t_{1/2\beta}$ (min)	CL (L/h/kg)	V_{dss} (L/kg)
	iv	25	3095				94	0.88 ± 0.09	1.58
fed	po	25	1009	32.6 ± 0.06	4.02 ± 0.72	90			
fasted[b]	po	25	1149	37.1 ± 0.08	5.30 ± 1.13	60			

[a] The values represent the mean ± standard error of the mean for six dogs. [b] The animals were fasted overnight. F (%) was determined by comparing the mean areas under the curves (AUC) after intravenous and oral doses. CL, plasma clearance rate; V_{dss}, volume of distribution; $t_{1/2\beta}$, plasma half-life; C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; F (%), percent bioavailability via oral route.

profile of JE-2147 was different from that of other HIV protease inhibitors that have been approved for marketing.[26,27]

Conclusion

In summary, we designed and synthesized a series of novel dipeptide HIV protease inhibitors containing Apns-Thz based on the transition-state isostere concept. From our SAR study on HIV protease inhibition by three different types of compounds, a combination of Dmt (P1′) and benzylamide group (P2′) led to an increase in the inhibitory activity of HMC-type compounds. Among them, **21f** (JE-2147) and **21i** (JE-1520) were highly potent inhibitors of HIV-1 protease with K_i values of 0.16−0.33 nM and also exerted a potent antiviral activity against HIV-1 IIIB-infected CEM cells with IC_{50} values ranging from 7 to 11 nM. Furthermore, the additional evidence of the pharmacokinetic profiles of these compounds indicated that **21f** (JE-2147) is an orally available and thus favorable HIV protease inhibitor.

Experimental Section

HIV Protease Inhibition. HIV protease substrate (SQNY-PIV) was synthesized by solid-phase methods using an Applied Biosystems model 430A. Recombinant HIV-1 protease (NY5-type sequence) was expressed in *Escherichia coli* and purified to a single band on sodium dodecyl sulfate−polyacrylamide gel electrophpresis.

In the inhibition assay, 25 μL of 200 mM 2-(*N*-morpholino-)ethanesulfonic acid (MES)−NaOH buffer, pH 6.0, containing 2 mM dithiothreitol (DTT) and 2 mM EDTA-2Na was mixed with 5 μL of inhibitor (50 nM or 0.5 μM) dissolved in DMSO and 10 μL of titrated HIV-1 protease (10.5 nM) in 50 mM MES−NaOH, pH 6.0, containing 2.5 mM DTT, 1 mM EDTA-2Na, 0.2% Nonidet P-40, and 15% glycerol. The mixture of protease and inhibitor was preincubated for 5 min at 37 °C, and the enzymic reaction was initiated by addition of 10 μL of a 75 mM substrate solution in the above-described assay buffer. After incubation for 60 min at 37 °C, the reaction was terminated by addition of 75 μL of 4% trifluoroacetic acid (TFA), and the C-terminal cleavage fragment (PIV) was separated by reverse-phase HPLC on a C_{18} column with linear gradient of water to acetonitrile (both solutions containing 0.1% TFA), detected by absorbance at 215 nm, and quantified by comparison with a synthetic product standard.

For some highly potent inhibitors, their K_i values were analyzed by a mathematical model for tight-binding inhibitors,[28] in which the concentration of inhibitor is less than or approximately equal to the enzyme concentration. The initial velocity data of HIV protease in the presence of various inhibitor concentrations were fitted by nonlinear regression analysis to eq 1 with KaleidaGraph (Version 3.08d) for Macintosh, where V is the initial velocity with an inhibitor, V_0 is the measured initial velocity in the absence of the inhibitor, the substrate K_m is estimated to be 21.4 mM, and S, Et, and It are the concentrations of substrate, active enzyme, and inhibitor, respectively.

$$V = \frac{V_0}{2Et}\left(\left\{\left[K_i\left(1+\frac{S}{K_m}\right)+It-Et\right]^2+4K_i\left(1+\frac{S}{K_m}\right)Et\right\}^{1/2}-\left[K_i\left(1+\frac{S}{K_m}\right)+It-Et\right]\right) \quad (1)$$

Antiviral Activity. Antiviral activity of test compounds was determined based on inhibition of HIV-1 IIIB-induced cytopathic effects in CEM-SS cells in vitro. The CEM-SS cells (2.5×10^4 cells/mL) were incubated in a total volume of 200 μL of tissue culture medium (RPMI-1640 medium plus 10% fetal calf serum with 50 μg of gentamicin/mL) containing test compound and HIV-1 IIIB for 6 days at 37 °C in a 5% CO_2 incubator. The virus was added to each well as a titer sufficient to give complete cell killing at 6 days postinfection. After incubation, HIV IIIB-induced cytopathic effects were analyzed by staining with the tetrazolium dye XTT.[29] The antiviral activity and cytotoxicity of a given compound are expressed as 50% inhibitory concentration (IC_{50}) and 50% toxic concentration (TC_{50}), respectively.

Pharmacokinetics. Pharmacokinetic parameters of the protease inhibitors were studied in rats and dogs. In the rat iv or id administration studies, three male Sprague−Dawley rats (300−400 g) received the compound at 10 mg/kg in 50% PEG (1 mL/kg) under anesthesia in combination with Ketaral (Sankyo Co., Ltd, Tokyo)/Selactal (Bayer AG, Germany). The iv administration was made via a femoral vein. In the id dosing study, rats were incised subphrenically for ca. 3 cm along the abdominal median line, a poly(ethylene) tube (Intramedic, PE10) was inserted into duodenum, and then the test solution was injected into the duodenum through the tube.

The pharmacokinetic behavior of JE-2147 in beagle dogs was evaluated following a single oral or iv dose. Eighteen male beagle dogs (7−10 kg; HRP, Inc., Cumberland, MD) were randomly assigned to one of three groups, with each group containing six animals. JE-2147 was administered intravenously as a bolus via the cephalic vein at a dose of 25 mg/kg (2 mL/kg) and orally by gavage at a dose of 25 mg/kg (5 mL/kg). JE-2147 was prepared as a 12.5 mg/mL solution in a 50% PEG-in-water vehicle.

In all of the animal studies, heparinized blood samples (0.5 mL) were obtained after dosing at appropriate times, and plasma (0.2 mL) was obtained by immediate centrifugation and kept frozen (−80 °C) until analyzed.

Sample Analysis. A plasma aliquot (0.2 mL) was combined with 4 mL of *tert*-butylmethyl ester containing an appropriate internal standard (0.5 mg/mL KNI-748, (*R*)-*N*-*tert*-butyl-3-[(2*S*,3*S*)-3-(4-carbamoyl-2,6-dimethylphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide). Samples were vortexed vigorously for 10 s and shakened for 1 h at room temperature followed by centrifugation at 2500*g* for 15 min at 4 °C. The organic layer (3.6 mL) was evaporated to dryness at 40 °C, and then samples were reconstituted in 0.3 mL of 50% methanol with vortexing. The analytical method for the quantification of JE-2147 in dog plasma samples was similar to that described above.

The parent inhibitors and a respective internal standard were separated from plasma contaminants on a Capcellpak C_{18} column (4.6 × 150 mm; Shiseido Ltd., Tokyo). The elution condition was a linear gradient of 45−60% acetonitrile in 0.1% TFA for 12 min at a flow rate of 1.0 mL/min with UV detection at 210 nm. The drug concentration in each plasma sample was calculated by the internal standard method. Standard plasma samples spiked with specified amounts of each compound were analyzed, and the calibration curve was prepared by plotting the concentration of test compound and its ratio to the internal standard.

The assays for each inhibitor were linear (correlation coefficients, >0.999) over the concentration range of 0−10 μg/mL, and the detection limit of quantification was 0.01 μg/mL.

Pharmacokinetic Analysis. Pharmacokinetic parameters for inhibitors were estimated by a noncompartmental method. Maximum plasma concentration (C_{max}), and time of maximum plasma concentration (T_{max}) were determined by inspection of individual subject concentration−time curves, and the mean area under the plasma concentration−time curve (AUC) was determined by the linear trapezoidal rule. The apparent plasma half-life ($t_{1/2\beta}$) was estimated from the slope of the terminal phase fitted to the log plasma concentration−time data by the method of least squares. The apparent distribution volume (V_{dss}) of the inhibitor was determined by eq 2:

$$V_{dss} = \text{dose iv} \times \text{AUMC}(0-\infty)/\text{AUC iv}(0-\infty) \quad (2)$$

where AUMC(0−∞) is the total area under the first moment of the drug concentration curve from zero to infinity. The

plasma clearance (CL) was calculated as the dose divided by the AUC from zero to infinity, $[AUC]_0^\infty$.

Chemistry. In general, reagents and solvents were used as purchased without further purification. Column chromatography was performed on Wakogel C-200 (Wako, 70−150 μm) or Wakogel C-300 (Wako, 45−75 μm). Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. Proton and carbon NMR spectra were recorded on a JEOL GSX270 FT NMR spectrometer. Chemical shifts are expressed in δ (ppm) from the internal standard tetramethylsilane. TOFMS were recorded on a Kompact MALDI III spectrometer. Elemental analyses were performed by the Toray Research Center and are within 0.4% of the calculated values unless otherwise noted.

Abbreviations: Boc, *tert*-butoxycarbonyl; Thz, (*R*)-1,3-thiazolidine-4-carbonyl; HOBt, 1-hydroxybenzotriazole; DCC, *N*,*N*′-dicyclohexylcarbodiimide; TOFMS, time-of-flight mass spectrometry; DMF, *N*,*N*-dimethylformamide; Apns, (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl; EDC, *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide; tBu, *tert*-butyl; DPP-Cl, diphenylphosphoryl chloride; HOSu, *N*-hydroxysuccinimide; THF, tetrahydrofuran; Dmt, (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carbonyl; DMSO, dimethyl sulfoxide; TMS-Cl, trimethylsilyl chloride.

(*R*)-*N*-*tert*-Butyl-1,3-thiazolidine-4-carboxamide (5a). To a solution of Boc-Thz-OH (11.65 g, 50 mmol) and HOBt (6.75 g, 50 mmol) in CH_2Cl_2 (80 mL) was added DCC (11.33 g, 55 mmol) in an ice-bath. After 30 min, *tert*-butylamine (13.75 mL, 150 mmol) in CH_2Cl_2 (60 mL) was dropped to the reaction mixture and stirred overnight. The reaction mixture was washed with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, and then evaporated. The residue was redissolved in CH_2Cl_2 (125 mL), and 4 N HCl in dioxane (125 mL) was added and stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O and filtrated. The filtrate was washed with CH_2Cl_2, adjusted to pH 8 with K_2CO_3, and extracted with CH_2Cl_2. After drying and concentrating, the obtained solid was recrystallized from *n*-hexane/toluene to give 7.87 g of the title compound, yield 84%: mp 63−65 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H), 2.75−2.82 (m, 1H), 2.91−2.98 (m, 1H), 3.10−3.21 (m, 1H), 3.62−3.71 (m, 1H), 4.01 (t, 1H, J = 9.7 Hz), 4.14 (t, 1H, J = 8.8 Hz), 7.56 (s, 1H); MS (TOF) m/z = 189 (M^+ + H). Anal. ($C_8H_{16}N_2OS$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (6a). To a solution of **5a** (1.05 g, 5.59 mmol) in DMF (10 mL) were added Boc-Apns-OH (1.50 g, 5.09 mmol), HOBt (0.69 g, 5.09 mmol), and EDC•HCl (1.07 g, 5.60 mmol), and the mixture was stirred overnight. To the reaction mixture were added CH_2Cl_2 and 1 N HCl, and then the organic layer was washed with 3% K_2CO_3 and brine, dried over $MgSO_4$, and evaporated to give Boc-Apns-Thz-NHtBu. To the solution of Boc-Apns-Thz-NHtBu in CH_2Cl_2 (13 mL) was added 4 N HCl in dioxane (13 mL), and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O, washed with CH_2Cl_2, and adjusted to pH 8 with K_2CO_3, to give a solid. The obtained solid was washed with hot methanol to give 1.57 g of the title compound, yield 85%: mp 208−210 °C; ^{1}H NMR (DMSO-d_6) δ 1.19 (s, 9H), 1.40 (br, 2H), 2.31−2.40 (m, 1H), 2.90 (t, 1H, J = 8.1 Hz), 3.01−3.07 (m, 2H), 3.16−3.26 (m, 1H), 4.11 (t, 1H, J = 7.6 Hz), 4.60 (d, 1H, J = 8.9 Hz), 4.77−4.82 (m, 1H), 4.89 (d, 1H, J = 8.6 Hz), 5.20 (d, 1H, J = 7.8 Hz), 7.16−7.31 (m, 5H), 7.57 (s, 1H); MS (TOF) m/z = 366 (M^+ + H). Anal. ($C_{18}H_{27}N_3O_3S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-benzoylamino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (8a). To a solution of compound **6a** (365 mg, 1.00 mmol), benzoic acid (122 mg, 1.00 mmol), and HOBt•H_2O (153 mg, 1.00 mmol) in DMF (3 mL) was added EDC•HCl (191 mg, 1.00 mmol) in an ice-bath, and the mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure and then redissolved in ethyl acetate. The organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. Purification of the crude product by silica gel column chromatography (CH_2Cl_2/methanol), and reprecipitation from *n*-hexane/ethyl acetate gave 296 mg of the title compound, yield 63%: mp 85−87 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H), 2.72−2.76 (m, 1H), 2.85−2.94 (m, 1H), 2.99−3.05 (m, 1H), 3.31−3.38 (m, 1H), 4.31−4.38 (m, 1H), 4.60−4.64 (m, 1H), 4.73−4.82 (m, 2H), 5.08 (d, 1H, J = 9.2 Hz), 5.22 (d, 1H, J = 6.8 Hz), 7.08−7.14 (m, 1H), 7.22 (t, 2H, J = 7.3 Hz), 7.40−7.53 (m, 5H), 7.70 (s, 1H), 7.81 (d, 1H, J = 7.0 Hz), 8.54 (d, 1H, J = 7.8 Hz); MS (TOF) m/z = 470 (M^+ + H). Anal. ($C_{25}H_{31}N_3O_4S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-(2-methylbenzoyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (8b). Compound **8b** was prepared from 2-methylbenzoic acid and compound **6a** in a manner similar to that described for compound **8a**, yield 61%: mp 90−92 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 9H), 2.05 (s, 3H), 2.7−2.9 (m, 2H), 2.98−3.05 (m, 1H), 3.28−3.33 (m, 1H), 4.3−4.5 (br, 1H), 4.55−4.65 (m, 1H), 4.7−4.9 (m, 2H), 5.06 (d, 1H, J = 9.4 Hz), 5.27 (d, 1H, J = 7.3 Hz), 7.14−7.27 (m, 7H), 7.40 (d, 2H, J = 6.8 Hz), 7.66 (s, 1H), 8.33 (d, 1H, J = 8.9 Hz); MS (TOF) m/z = 484 (M^+ + H). Anal. ($C_{26}H_{33}N_3O_4S$•0.25EtOAc) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxybenzoyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (9c). To a solution of 3-acetoxybenzoic acid (180 mg, 1.1 mmol) and triethylamine (167 μL, 1.2 mmol) in ethyl acetate (3 mL) was added DPP-Cl (228 μL, 1.1 mmol) in an ice-bath, and the mixture was stirred for 1 h. Then to the reaction mixture, compound **6a** (365 mg, 1.00 mmol) and triethylamine (209 μL, 1.5 mmol) were added in an ice-bath and stirred overnight. The reaction mixture was washed sequentially with 1 N HCl, 3% K_2CO_3, and brine and concentrated. To the solution of the resulting residue in methanol (5 mL), was added 1 N NaOH (1.5 mL, 1.5 mmol), and the mixture was stirred for 1 h. The mixture was acidified with 1 N HCl, and the aqueous phase was extracted with ethyl acetate. The organic extract was washed sequentially with 1 N HCl, 3% K_2CO_3, and brine, dried over $MgSO_4$, filtered, and concentrated. Purification of the crude product by silica gel column chromatography (CH_2Cl_2/methanol) and reprecipitation from *n*-hexane/ethyl acetate gave 321 mg of the title compound, yield 66%: mp 115−118 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 9H), 2.7−2.8 (m, 1H), 2.84−2.93 (m, 1H), 2.98−3.05 (m, 1H), 3.30−3.37 (m, 1H), 4.2−4.4 (br, 1H), 4.57−4.60 (m, 1H), 4.71−4.81 (m, 2H), 5.07 (d, 1H, J = 9.2 Hz), 5.20 (d, 1H, J = 7.0 Hz), 6.86−6.90 (m, 1H), 7.09−7.23 (m, 6H), 7.44 (d, 2H, J = 7.3 Hz), 7.68 (s, 1H), 8.42 (d, 1H, J = 8.4 Hz), 9.60 (s, 1H); MS (TOF) m/z = 486 (M^+ + H). Anal. ($C_{25}H_{31}N_3O_5S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (9d). Compound **9d** was prepared from 3-acetoxy-2-methylbenzoic acid and compound **6a** in a manner similar to that described for compound **9c**, yield 76%: mp 220−223 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H), 1.82 (s, 3H), 2.74 (m, 2H), 3.02 (m, 1H), 3.32 (m, 1H), 4.35 (bs, 1H), 4.58 (bs, 1H), 4.78 (m, 2H), 5.09 (d, 1H), 5.21 (d, 1H), 6.56 (d, 1H), 6.77 (d, 1H), 6.94 (t, 1H), 7.15 (t, 1H), 7.23 (t, 2H), 7.38 (d, 2H), 7.64 (s, 1H), 8.22 (d, 1H), 9.38 (s, 1H); MS (TOF) m/z = 500 (M^+ + H). Anal. ($C_{26}H_{33}N_3O_5S$•0.25 H_2O) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(3-amino-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (8e). Compound **8e** was prepared from 3-amino-2-methylbenzoic acid and compound **6a** in a manner similar to that described for compound **8a**, yield 31%: mp 114−117 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H), 1.72 (s, 3H), 2.7−2.8 (m, 2H), 2.98−3.05 (m, 1H), 3.28−3.33 (m, 1H), 4.3−4.4 (m, 1H), 4.5−4.6 (m, 1H), 4.7−4.9 (m, 4H), 5.05 (d, 1H, J = 9.2 Hz), 5.19 (d, 1H, J = 7.3 Hz), 6.33 (d, 1H, J = 7.6 Hz), 6.60 (d, 1H, J = 7.3 Hz), 6.84 (t, 1H, J = 7.8 Hz), 7.16−7.26 (m, 3H), 7.39 (d, 1H, J = 6.8 Hz), 7.66 (s, 1H), 8.16 (d, 1H, J = 8.1 Hz); MS (TOF) m/z = 499 (M^+ + H). Anal. ($C_{26}H_{34}N_4O_4S$•0.5 H_2O) Calcd: C, 61.51; H, 6.95; N, 11.04. Found: C, 61.00; H, 6.96; N, 10.74.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(2-ethyl-3-hydroxybenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (9f). Compound **9f** was prepared from 3-acetoxy-2-ethylbenzoic acid and compound **6a** in a manner similar to that described for compound **9c**, yield 62%: mp 216−218 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, J = 7.5 Hz), 1.25 (s, 9H), 2.35 (m, 2H), 2.74 (m, 2H), 3.00 (m, 1H), 3.2−3.4 (m, 1H), 4.35 (bs, 1H), 4.55 (bs, 1H), 4.77 (m, 2H), 5.05 (d, 1H, J = 9.3 Hz), 5.25 (d, 1H, J = 7.1 Hz), 6.55 (d, 1H, J = 7.1 Hz), 6.78 (d, 1H, J = 8.6 Hz), 6.94 (dd, 1H, J = 7.9 Hz, 7.9 Hz), 7.18 (d, 1H, J = 6.6 Hz), 7.22 (t, 2H, J = 7.1 Hz), 7.38 (d, 2H, J = 7.5 Hz), 7.63 (s, 1H), 8.21 (d, 1H, J = 7.9 Hz), 9.31 (s, 1H); MS (TOF) m/z = 514 (M^+ + H). Anal. ($C_{27}H_{35}N_3O_5S$•0.25 H_2O) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)- 2-hydroxy-3-(3-hydroxy-2-propylbenzoyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (9g). Compound **9g** was prepared from 3-acetoxy-2-propylbenzoic acid and compound **6a** in a manner similar to that described for compound **9c**, yield 25%: mp 205−207 °C; ^{1}H NMR (DMSO-d_6) δ 0.69 (t, 3H, J = 7.0 Hz), 1.2−1.4 (br, 2H), 1.26 (s, 9H), 2.2−2.3 (m, 1H), 2.3−2.4 (m, 1H), 2.7−2.8 (m, 1H), 3.0−3.1 (m, 1H), 3.28−3.34 (m, 1H), 4.2−4.4 (m, 1H), 4.5−4.6 (m, 1H), 4.75−4.82 (m, 2H), 5.02 (d, 1H, J = 9.4 Hz), 5.18 (d, 1H, J = 7.3 Hz), 6.57 (d, 1H, J = 7.3 Hz), 6.78 (d, 1H, J = 7.5 Hz), 6.96 (t, 1H, J = 7.7 Hz), 7.15−7.26 (m, 3H), 7.40 (d, 1H, J = 7.3 Hz), 7.66 (s, 1H), 8.23 (d, 1H, J = 8.4 Hz), 9.29 (s, 1H); MS (TOF) m/z = 528 (M^+ + H). Anal. ($C_{28}H_{37}N_3O_5S$•0.5H_2O) C, H, N.

(*R*)-*N*-*tert*-Butyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (5b). To a solution of Boc-Dmt-OH (15.0 g, 58.37 mmol) and HOSu (7.38 g, 64.21 mmol) in THF (50 mL) was added DCC (13.23 g, 64.21 mmol) in THF (50 mL) in an ice-bath. After 60 min, *tert*-butylamine (30.64 mL, 291.85 mmol) in THF (100 mL) was dropped into the reaction mixture and stirred overnight. The reaction mixture was filtered, and the filtrate was evaporated. The residue was redissolved in ethyl acetate, washed with 5% citric acid, 3% K_2CO_3, and brine, dried over $MgSO_4$, and evaporated. The residue was redissolved in CH_2Cl_2 (146 mL); 4 N HCl in dioxane (146 mL) was added and stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O and filtered. The filtrate was washed with CH_2Cl_2, adjusted to pH 8 with K_2CO_3, and extracted with CH_2Cl_2. After drying and concentrating, the obtained solid was recrystallized from *n*-heptane to give 9.80 g of the title compound, yield 79%: mp 75−77 °C; ^{1}H NMR (DMSO-d_6) δ 1.16 (s, 3H), 1.27 (s, 9H), 1.52 (s, 3H), 3.16 (d, 1H, J = 13.2 Hz), 3.46−3.58 (m, 1H), 3.99 (dd, 1H, J = 11.8 Hz, 9.2 Hz), 4.26 (dd, 1H, J = 7.3 Hz, 9.2 Hz), 7.47 (s, 1H); MS (TOF) m/z = 217 (M^+ + H). Anal. ($C_{10}H_{20}N_2OS$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6b). To a solution of **5b** (3.00 g, 14.15 mmol) in DMF (30 mL) were added Boc-Apns-OH (4.17 g, 14.15 mmol), HOBt (1.91 g, 14.15 mmol), and DCC (3.21 g, 15.57 mmol), and the mixture was stirred overnight. To the reaction mixture were added toluene (100 mL) and 5% citric acid, and then the solution was filtrated. The organic layer was washed with 3% K_2CO_3 and brine, dried over $MgSO_4$, and evaporated. The residue was redissolved in CH_2Cl_2 (18 mL); 4 N HCl in dioxane (18 mL) was added and stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O and filtrated. The filtrate was washed with CH_2Cl_2, adjusted to pH 8 with K_2CO_3, and extracted with CH_2Cl_2. After drying with $MgSO_4$ and concentrating, the obtained solid was recrystallized from *n*-heptane/ethanol to give 3.60 g of the title compound, yield 65%: mp 177−180 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (s, 9H), 1.35 (s, 3H), 1.3−1.5 (m, 2H), 1.49 (s, 3H), 2.30−2.38 (m, 1H), 2.88−3.04 (m, 2H), 4.10 (t, 1H, J = 7.3 Hz), 4.36 (s, 1H), 4.90 (s, 1H), 5.19 (d, 1H, J = 7.3 Hz), 7.16−7.31 (m, 5H), 7.52 (s, 1H); MS (TOF) m/z = 394 (M^+ + H). Anal. ($C_{20}H_{31}N_3O_3S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (9h). Compound **9h** was prepared from 3-acetoxy-2-methylbenzoic acid and compound **6b** in a manner similar to that described for compound **9c**, yield 67%: mp 203−205 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 1.80 (s, 3H), 2.75 (m, 2H), 3.2−3.4 (m, 1H), 4.35 (bs, 1H), 4.52 (bs and s, 2H), 4.98 (d, 1H), 5.18 (d, 1H), 5.27 (d, 1H), 6.55 (d, 1H), 6.76 (d, 1H), 6.94 (t, 1H), 7.13 (t, 1H), 7.23 (t, 2H), 7.36 (d, 2H), 7.63 (s, 1H), 8.22 (d, 1H), 9.36 (s, 1H); MS (TOF) m/z = 528 (M^+ + H). Anal. ($C_{28}H_{37}N_3O_5S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(2-ethyl-3-hydroxybenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (9i). Compound **9i** was prepared from 3-acetoxy-2-ethylbenzoic acid and compound **6b** in a manner similar to that described for compound **9c**, yield 88%: mp 144−146 °C; ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, J = 7.3 Hz), 1.27 (s, 9H), 1.41 (s, 3H), 1.49 (s, 3H), 2.3−2.6 (m, 2H), 2.7−2.9 (m, 2H), 4.3−4.4 (m, 1H), 4.53 (s, 2H), 4.97 (d, 1H, J = 8.9 Hz), 5.17 (d, 1H, J = 8.6 Hz), 5.26 (d, 1H, J = 7.0 Hz), 6.55 (d, 1H, J = 7.8 Hz), 6.78 (d, 1H, J = 7.8 Hz), 6.95 (t, 1H, J = 7.6 Hz), 7.16−7.26 (m, 3H), 7.39 (d, 1H, J = 6.8 Hz), 7.64 (s, 1H), 8.22 (d, 1H, J = 8.9 Hz), 9.33 (s, 1H); MS (TOF) m/z = 542 (M^+ + H). Anal. ($C_{29}H_{39}N_3O_5S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-(phenoxyacetyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (12a). To a solution of compound **6a** (365 mg, 1.00 mmol), phenoxyacetic acid (167 mg, 1.10 mmol), and HOBt•H_2O (153 mg, 1.00 mmol) in DMF (3 mL) was added EDC•HCl (210 mg, 1.10 mmol) in an ice bath, and the mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure and then redissolved in ethyl acetate. The organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. Purification of the crude product by silica gel column chromatography (CH_2Cl_2/methanol) and reprecipitation from *n*-hexane/ethyl acetate gave 300 mg of the title compound, yield 60%: mp 68−69 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H), 2.6−2.8 (m, 2H), 2.95−3.02 (m, 1H), 3.29−3.36 (m, 1H), 4.1−4.3 (m, 1H), 4.39 (s, 2H), 4.48−4.52 (m, 1H), 4.62 (d, 1H, J = 9.2 Hz), 4.77 (t, 1H, J = 7.2 Hz), 4.96 (d, 1H, J = 9.2 Hz), 5.29 (d, 1H, J = 6.8 Hz), 6.77 (d, 2H, J = 8.4 Hz), 6.93 (t, 1H, J = 7.4 Hz), 7.17−7.25 (m, 5H), 7.34 (d, 2H, J = 6.8 Hz), 7.70 (s, 1H), 8.24 (d, 1H, J = 8.4 Hz); MS (TOF) m/z = 500 (M^+ + H). Anal. ($C_{26}H_{33}N_3O_5S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-(2-methylphenoxyacetyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (12b). Compound **12b** was prepared from 2-methylphenoxyacetic acid and compound **6a** in a manner similar to that described for compound **12a**, yield 39%: mp 69−72 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H), 2.19 (s, 3H), 2.6−2.8 (m, 2H), 2.96−3.02 (m, 1H), 3.29−3.36 (m, 1H), 4.1−4.3 (m, 1H), 4.41 (d, 2H, J = 5.9 Hz), 4.47−4.51 (m, 1H), 4.60 (d, 1H, J = 9.5 Hz), 4.76 (t, 1H, J = 7.0 Hz), 4.96 (d, 1H, J = 9.2 Hz), 5.33 (d, 1H, J = 7.0 Hz), 6.58 (d, 1H, J = 7.8 Hz), 6.83 (t, 1H, J = 7.3 Hz), 6.99−7.02 (m, 1H), 7.12−7.26 (m, 4H), 7.34 (d, 2H, J = 7.0 Hz), 7.70 (s, 1H), 8.17 (d, 1H, J = 8.4 Hz); MS (TOF) m/z = 514 (M^+ + H). Anal. ($C_{27}H_{35}N_3O_5S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-(3-methylphenoxyacetyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (12c). Compound **12c** was prepared from 3-methylphenoxyacetic acid and compound **6a** in a manner similar to that described for compound **12a**, yield 53%: mp 59−60 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H), 2.24 (s, 3H), 2.6−2.8 (m, 2H), 2.95−3.02 (m, 1H), 3.2−3.4 (m, 1H), 4.1−4.3 (m, 1H), 4.37 (s, 2H), 4.48−4.52 (m, 1H), 4.61 (d, 1H, J = 9.2 Hz), 4.77 (t, 1H, J = 7.0 Hz), 4.96 (d, 1H, J = 9.2 Hz), 5.29 (d, 1H, J = 7.3 Hz), 6.59 (m, 1H), 6.67 (s, 1H), 6.75 (d, 1H, J = 7.3 Hz), 7.07−7.24 (m, 4H), 7.33 (d, 2H, J = 6.5 Hz), 7.70 (s, 1H), 8.19 (d, 1H, J = 8.1 Hz); MS (TOF) m/z = 514 (M^+ + H). Anal. ($C_{27}H_{35}N_3O_5S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-(4-methylphenoxyacetyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (12d). Compound **12d** was prepared from 4-methylphenoxyacetic acid and compound **6a** in a manner similar to that described for compound **12a**, yield 80%: mp 68−70 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H), 2.22 (s, 3H),

2.6−2.8 (m, 2H), 2.95−3.02 (m, 1H), 3.29−3.36 (m, 1H), 4.1−4.3 (m, 1H), 4.35 (s, 2H), 4.48−4.52 (m, 1H), 4.61 (d, 1H, J = 9.5 Hz), 4.77 (t, 1H, J = 7.0 Hz), 4.96 (d, 1H, J = 9.2 Hz), 5.29 (d, 1H, J = 7.6 Hz), 6.68 (d, 2H, J = 8.4 Hz), 7.02 (d, 2H, J = 8.4 Hz), 7.16−7.25 (m, 3H), 7.33 (d, 2H, J = 6.5 Hz), 7.70 (s, 1H), 8.19 (d, 1H, J = 8.6 Hz); MS (TOF) m/z = 514 (M^+ + H). Anal. ($C_{27}H_{35}N_3O_5S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(2,6-dimethylphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (12e). Compound **12e** was prepared from 2,6-dimethylphenoxyacetic acid and compound **6a** in a manner similar to that described for compound **12a**, yield 66%: mp 81−83 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.26 (S, 9H), 2.15 (S, 6H), 2.76−2.78 (m, 2H), 3.02 (dd, 1H, J = 7.1 Hz, 10.6 Hz), 3.2−3.4 (m, 1H), 3.98 (d, 1H, J = 14.3 Hz), 4.18 (d, 1H, J = 14.3 Hz), 4.3−4.4 (m, 1 H), 4.5−4.6 (br, 1H), 4.63 (d, 1H, J = 9.6 Hz), 4.77 (t, 1H, J = 7.1 Hz), 5.00 (d, 1H, J = 9.6 Hz), 5.33 (d, 1H, J = 6.6 Hz), 6.92 (m, 1H), 7.00 (d, 2H, J = 6.6 Hz), 7.1−7.3 (m, 3H), 7.36 (d, 2H, J = 7.5 Hz), 7.68 (s, 1H), 8.11(d, 1H, J = 8.8 Hz); MS (TOF) m/z = 528 (M^+ + H). Anal. ($C_{28}H_{37}N_3O_5S$) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)- 3-(2-ethyl-6-methylphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (12f). Compound **12f** was prepared from 2-ethyl-6-methylphenoxyacetic acid and compound **6a** in a manner similar to that described for compound **12a**, yield 42%: mp 72−74 °C; ^{1}H NMR (DMSO-d_6) δ 1.12 (t, 3H, J = 7.3 Hz), 1.26 (s, 9H), 2.49−2.57 (m, 2H), 2.7−2.8 (m, 2H), 2.98−3.05 (m, 1H), 3.30−3.37 (m, 1H), 3.98 (d, 1H, J = 14.0 Hz), 4.18 (d, 1H, J = 14.0 Hz), 4.2−4.4 (m, 1H), 4.51−4.54 (m, 1H), 4.63 (d, 1H, J = 9.2 Hz), 4.77 (t, 1H, J = 7.0 Hz), 5.01 (d, 1H, J = 9.4 Hz), 5.34 (d, 1H, J = 7.3 Hz), 6.98−7.06 (m, 3H), 7.17−7.27 (m, 3H), 7.37 (d, 2H, J = 7.0 Hz), 7.70 (s, 1H), 8.10 (d, 1H, J = 8.9 Hz); MS (TOF) m/z = 542 (M^+ + H). Anal. ($C_{29}H_{39}N_3O_5S$•0.5EtOAc) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-(2-methyl-6-propylphenoxyacetyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (12g). Compound **12g** was prepared from 2-methyl-6-propylphenoxyacetic acid and compound **6a** in a manner similar to that described for compound **12a**, yield 84%: mp 68−71 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7.3 Hz), 1.26 (s, 9H), 1.50−1.58 (m, 2H), 2.14 (s, 3H), 2.47−2.53 (m, 2H),, 2.7−2.9 (m, 2H), 2.98−3.05 (m, 1H), 3.2−3.4 (m, 1H), 3.99 (d, 1H, J = 14.0 Hz), 4.17 (d, 1H, J = 14.0 Hz), 4.2−4.4 (m, 1 H), 4.5−4.6 (br, 1H), 4.62 (d, 1H, J = 9.2 Hz), 4.77 (t, 1H, J = 7.0 Hz), 5.01 (d, 1H, J = 9.2 Hz), 5.35 (d, 1H, J = 7.0 Hz), 6.96−7.04 (m, 3H), 7.17−7.27 (m, 3H), 7.36 (d, 2H, J = 6.8 Hz), 7.70 (s, 1H), 8.11(d, 1H, J = 8.6 Hz); MS (TOF) m/z = 556 (M^+ + H). Anal. ($C_{30}H_{41}N_3O_5S$•0.5EtOAc) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(2,6-diethylphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (12h). Compound **12h** was prepared from 2,6-diethylphenoxyacetic acid and compound **6a** in a manner similar to that described for compound **12a**, yield 63%: mp 65−67 °C; ^{1}H NMR (DMSO-d_6) δ 1.12 (t, 6H, J = 7.6 Hz), 1.26 (s, 9H), 2.49−2.57 (m, 4H), 2.7−2.8 (m, 2H), 2.98−3.05 (m, 1H), 3.30−3.37 (m, 1H), 3.97 (d, 1H, J = 14.0 Hz), 4.17 (d, 1H, J = 14.0 Hz), 4.3−4.4 (m, 1H), 4.5−4.6 (m, 1H), 4.64 (d, 1H, J = 9.2 Hz), 4.77 (t, 1H, J = 7.0 Hz), 5.02 (d, 1H, J = 9.2 Hz), 5.34 (d, 1H, J = 7.3 Hz), 7.05 (s, 3H), 7.18−7.28 (m, 3H), 7.36 (d, 2H, J = 7.0 Hz), 7.69 (s, 1H), 8.09 (d, 1H, J = 8.9 Hz); MS (TOF) m/z = 556 (M^+ + H). Anal. ($C_{30}H_{41}N_3O_5S$• 0.5EtOAc) C, H, N.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(2,6-dimethylphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12i). Compound **12i** was prepared from 2,6-dimethylphenoxyacetic acid and compound **6b** in a manner similar to that described for compound **12a**, yield 92%: mp 87−89 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (S, 9H), 1.40 (S, 3H), 1.49 (S, 3H), 2.14 (S, 6H), 2.7−2.9 (m, 2H), 3.98 (d, 1H, J = 14.1 Hz), 4.19 (d, 1H, J = 14.1 Hz), 4.3−4.4 (m, 1H), 4.4−4.5 (br, 1H), 4.52 (S, 1H), 4.92 (d, 1H, J = 10.2 Hz), 4.97 (d, 1H, J = 10.2 Hz), 5.34 (d, 1H, J = 7.2 Hz), 6.92 (m, 1H), 7.00 (d, 2H, J = 6.6 Hz), 7.1−7.3 (m, 3H), 7.35 (d, 2H, J = 7.5 Hz), 7.66 (s, 1H), 8.11(d, 1H, J = 8.8 Hz); MS (TOF) m/z = 556 (M^+ + H). Anal. ($C_{30}H_{41}N_3O_5S$•0.5EtOAc) C, H, N.

2,6-Dimethylbenzyl Alcohol (14). To a solution of 2,6-dimethylbenzoic acid (1.50 g, 10.0 mmol) and K_2CO_3 (1.38 g, 10 mmol), in DMF (10 mL), was added methyl iodide (1.24 mL, 20 mmol) in an ice bath, and the mixture was stirred overnight. To the reaction mixture were added toluene and water, and the organic layer was washed with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. The oily residue was redissolved in THF (20 mL), added to $LiAlH_4$ (0.57 g, 15 mmol), and stirred for 4 h in an ice bath. To the reaction mixture were added 1 N HCl and ethyl acetate, and the organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was recrystallized from *n*-hexane to give 0.88 g of the title compound, yield 65%: mp 78−80 °C; ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 6H), 4.47 (d, 2H, J = 5.1 Hz), 4.67 (t, 1H, J = 5.1 Hz), 6.96 (d, 2H, J = 6.6 Hz), 7.03 (dd, 1H, J = 6.6 Hz). Anal. ($C_9H_{12}O$) C, H.

***N*-(2,6-Dimethylbenzyl)phthalimide (15).** To a solution of compound **14** (0.68 g, 5.0 mmol) in DMSO (1.77 mL) was added TMS-Cl (1.90 mL, 15 mmol), and the mixture was stirred for 30 min. To the reaction mixture were added ethyl acetate and water and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The oily residue was redissolved in DMF (10 mL), added to potassium phthalimide (1.11 g, 6.0 mmol), and stirred overnight. To the reaction mixture was added ethyl acetate and organic phase was washed with 3% Na_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was recrystallized from *n*-hexane to give 0.90 g of the title compound, yield 68%: mp 158−160 °C; ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 6H), 4.47 (s, 2H), 6.9−7.1 (m, 3H), 7.82 (s, 4H). Anal. ($C_{17}H_{15}NO_2$) C, H, N.

(*R*)-*N*-(2,6-Dimethylbenzyl)-1,3-thiazolidine-4-carboxamide (18a). To a solution of compound **14** (0.90 g, 3.40 mmol) in ethanol (10 mL) was added hydrazine monohydrate (0.25 mL, 5.09 mmol), and the mixture was stirred for 3 h under reflux condition. To the reaction mixture was added c HCl to pH 1, and the mixture was stirred for 2 h under the same conditions. After water was added and filtered, the filtrate was evaporated and adjusted to pH 10 with 2 N NaOH and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The oily residue was redissolved in CH_2Cl_2 (10 mL), added to Boc-Thz-OH (0.87 g, 3.74 mmol), HOBt (0.50 g, 3.74 mmol), and EDC•HCl (0.78 g, 4.08 mmol), and stirred overnight. The reaction mixture was washed with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/methanol). The oily residue was redissolved in CH_2Cl_2 (10 mL), added to 4 N HCl in dioxane (10 mL), and stirred for 3 h. The reaction mixture was concentrated under reduced pressure and then redissolved CH_2Cl_2. The organic phase was washed with 3% K_2CO_3 and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was recrystallized from *n*-hexane to give 0.54 g of the title compound, yield 64%: mp 146−148 °C; ^{1}H NMR (DMSO-d_6) δ 2.30 (S, 6H), 2.74−2.81 (m, 1H), 2.93−2.99 (m, 1H), 3.1−3.3 (br, 1H), 3.6−3.8 (br, 1H), 3.98 (t, 1H, J = 9.7 Hz), 4.14 (t, 1H, J = 8.4 Hz), 4.2−4.4 (m, 2H), 7.00−7.11 (m, 3H), 8.03 (br, 1H); MS (TOF) m/z = 251 (M^+ + H). Anal. ($C_{13}H_{18}N_2OS$) C, H, N.

(*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (19a). To a solution of compound **18a** (0.23 g, 1.05 mmol) were added Boc-Apns-OH (0.30 g, 1.00 mmol), HOBt (0.14 g, 1.00 mmol) in ethyl acetate (5 mL), and DCC (0.22 g, 1.10 mmol) in an ice bath, and the mixture was stirred overnight. The reaction mixture was filtrated, washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. The oily residue was redissolved in CH_2Cl_2 (5 mL), added to 4 N HCl in dioxane (5 mL), and stirred for 3 h. The reaction mixture was concentrated under reduced pressure and then redissolved in water. The aqueous phase

was filtered, washed with toluene, neutralized with 2 N NaOH, and extract with CH_2Cl_2. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was recrystallized from *n*-hexane/ethyl acetate gave 0.20 g of the title compound, yield 45%: mp 150−153 °C; ^{1}H NMR (DMSO-d_6) δ 0.6−0.8 (br, 2H), 2.10 (s, 6H), 2.1−2.3 (m, 1H), 2.40−2.51 (m, 1H), 3.07−3.16 (m, 2H), 3.21−3.33 (m, 1H), 3.97 (t, 1H, J = 8.8 Hz), 4.12 (d, 2H, J = 4.1 Hz), 4.70 (s, 2H), 4.86−4.89 (m, 1H), 5.26 (d, 1H, J = 8.9 Hz), 6.62−6.71 (m, 3H), 7.05 (d, 2H, J = 6.8 Hz), 7.27−7.37 (m, 3H), 8.37 (s, 1H); MS (TOF) m/z = 428 (M^+ + H). Anal. ($C_{23}H_{29}N_3O_3S$) C, H, N.

(*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (21a). Compound **21a** was prepared from 3-acetoxy-2-methylbenzoic acid and compound **19a** in a manner similar to that described for compound **9c**, yield 83%: mp 124−127 °C; ^{1}H NMR (DMSO-d_6) δ 1.83 (s, 3H), 2.30 (s, 6H), 2.7−2.8 (m, 2H), 2.9−3.1 (m, 1H), 3.29−3.36 (m, 1H), 4.21−4.27 (m, 1H), 4.3−4.5 (m, 2H), 4,5−4.6 (br, 1H), 4.7−4.9 (m, 2H), 5.07 (d, 1H, J = 9.2 Hz), 5.23 (d, 1H, J = 6.8 Hz), 6.57 (d, 1H, J = 7.3 Hz), 6.79 (d, 1H, J = 7.8 Hz), 6.93−7.07 (m, 4H), 7.17−7.29 (m, 3H), 7.40 (d, 2H, J = 6.8 Hz), 8.15 (t, 1H, J = 4.9 Hz), 8.25 (d, 1H, J = 8.6 Hz), 9.39 (s, 1H); MS (TOF) m/z = 562 (M^+ + H). Anal. ($C_{31}H_{35}N_3O_5S$•0.5H_2O) C, H, N.

(*R*)-*N*-Benzyl-1,3-thiazolidine-4-carboxamide (18b). To a solution of Boc-Thz-OH (2.33 g, 10.0 mmol), benzylamine (1.20 mL, 11.0 mmol), and HOBt•H_2O (1.53 g, 10.0 mmol) in CH_2Cl_2 (30 mL) was added EDC•HCl (2.10 g, 11.0 mmol) in an ice bath, and the mixture was stirred for 2 h. The reaction mixture was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. The oily residue was redissolved in CH_2Cl_2 (25 mL), added to 4 N HCl in dioxane (25 mL), and stirred for 3 h. The reaction mixture was concentrated under reduced pressure and then redissolved in water. The aqueous phase was washed with CH_2Cl_2, neutralized with K_2CO_3, and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was recrystallized from *n*-hexane/ethyl acetate gave 1.75 g of the title compound, yield 79%: mp 80−82 °C; ^{1}H NMR (DMSO-d_6) δ 3.23−3.39 (m, 1H), 3.86−3.94 (m, 1H), 4.10 (d, 2H, J = 9.7 Hz), 4.22−4.37 (m, 3H), 7.20−7.37 (m, 5H), 8.55 (br, 1H); MS (TOF) m/z = 223 (M^+ + H). Anal. ($C_{11}H_{14}N_2OS$) C, H, N.

(*R*)-*N*-Benzyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (19b). To a solution of compound **18b** (0.73 g, 3.3 mmol) were added Boc-Apns-OH (0.89 g, 3.0 mmol), HOBt (0.41 g, 3.0 mmol) in DMF (15 mL), and EDC•HCl (0.63 g, 3.3 mmol) in an ice bath, and the mixture was stirred overnight. To the reaction mixture was added ethyl acetate, and the organic phase was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. The oily residue was redissolved in CH_2Cl_2 (25 mL), added to 4 N HCl in dioxane (25 mL), and stirred for 3 h. The reaction mixture was concentrated under reduced pressure and then redissolved in water. The aqueous phase was washed with toluene, neutralized with 2 N NaOH, and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated. Purification of the crude product by silica gel column chromatography (CH_2Cl_2/methanol) and recrystallization from *n*-hexane/ethyl acetate gave 0.40 g of the title compound, yield 33%: mp 91−93 °C; ^{1}H NMR (DMSO-d_6) δ 1.3−1.6 (br, 2H), 2.29−2.38 (m, 1H), 2.75−2.82 (m, 1H), 3.13−3.34 (m, 3H), 4.10 (t, 1H, J = 7.3 Hz), 4.16−4.31 (m, 2H), 4.77 (d, 1H, J = 8.9 Hz), 4.83 (d, 1H, J = 8.9 Hz), 4.95−4.99 (m, 1H), 5.37 (d, 1H, J = 7.8 Hz), 7.09−7.31 (m, 10H), 9.02 (t, 1H, J = 5.9 Hz); MS (TOF) m/z = 400 (M^+ + H). Anal. ($C_{21}H_{25}N_3O_3S$) C, H, N.

(*R*)-*N*-Benzyl-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (21b). Compound **21b** was prepared from 3-acetoxy-2-methylbenzoic acid and compound **19b** in a manner similar to that described for compound **9c**, yield 56%: mp 115−117 °C; ^{1}H NMR (DMSO-d_6) δ 1.83 (s, 3H), 2.7−2.9 (m, 2H), 3.07−3.14 (m, 1H), 3.31−3.38 (m, 1H), 4.1−4.5 (m, 4H), 4.78−4.88 (m, 2H), 5.05 (d, 1H, J = 9.2 Hz), 5.50 (d, 1H, J = 7.0 Hz), 6.56 (d, 1H, J = 7.3 Hz), 6.78 (d, 1H, J = 7.3 Hz), 6.94 (t, 1H, J = 7.3 Hz), 7.16−7.34 (m, 10H), 8.14 (d, 1H, J = 8.4 Hz), 8.45 (br, 1H), 9.39 (s, 1H); MS (TOF) m/z = 534 (M^+ + H). Anal. ($C_{29}H_{31}N_3O_5S$•0.5H_2O) C, H, N.

(*R*)-*N*-(2-Methylbenzyl)-1,3-thiazolidine-4-carboxamide (18c). Compound **18c** was prepared from Boc-Thz-OH and 2-methylbenzylamine in a manner similar to that described for compound **18b**, yield 85%: mp 114−116 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (S, 3H), 2.85−3.05 (m, 3H), 3.2−3.4 (m, 1H), 3.86 (m, 1H), 4.0−4.2 (m, 2H), 4.2−4.3 (br, 2H), 7.0−7.2 (br, 4H), 8.34 (br, 1H); MS (TOF) m/z = 237 (M^+ + H). Anal. ($C_{12}H_{16}N_2OS$) C, H, N.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (19c). Compound **19c** was prepared from Boc-Apns-OH and compound **18c** in a manner similar to that described for compound **19b**, yield 52%: mp 144−146 °C; ^{1}H NMR ($CDCl_3$) δ 0.2−1.4 (br, 2H), 2.20 (s, 3H), 2.2−2.4 (m, 1H), 2.4−2.6 (m, 1H), 3.14 (d, 2H, J = 16.8 Hz), 3.21 (t, 1H, J = 5.4 Hz), 3.48 (d, 1H, J = 9.6 Hz), 3.94 (d, 1H, J = 9.6 Hz), 4.1−4.3 (m, 1H), 4.3−4.5 (m, 1H), 4.55 (d, 1H, J = 7.5 Hz), 6.8−7.1 (m, 3H), 7.9−8.1 (br, 1H); MS (TOF) m/z = 414 (M^+ + H). Anal. ($C_{22}H_{27}N_3O_3S$•0.25H_2O) C, H, N.

(*R*)-*N*-(2-Methylbenzyl)-3-{(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (21c). Compound **21c** was prepared from 3-acetoxy-2-methylbenzoic acid and compound **19c** in a manner similar to that described for compound **9c**, yield 91%: mp 112−115 °C; ^{1}H NMR (DMSO-d_6) δ 1.83 (s, 3H), 2.23 (s, 3H), 2.78 (m, 2H), 3.10 (m, 1H), 3.2−3.4 (m, 1H), 4.18 (d, 1H, J = 6.0 Hz), 4.30 (d, 1H, J = 7.1 Hz), 4.38 (m, 2H), 4.78 (d, 1H, J = 8.7 Hz), 4.87(t, 1H, J = 6.4 Hz), 5.03 (d, 1H, J = 10.0 Hz), 5.45 (d, 1H, J = 6.4 HZ), 6.55 (d, 1H, J = 7.2 Hz), 6.77 (d, 1H, J = 8.1 Hz), 6.93 (dd, 1H, J = 6.4 Hz), 7.12 (br, 4H), 7.23(br, 3H), 7.32 (d, 2H, J = 6.0 Hz), 8.15 (d, 1H, J = 7.9 Hz), 8.35 (br, 1H), 9.37 (s, 1H); MS (TOF) m/z = 548 (M^+ + H). Anal. ($C_{30}H_{33}N_3O_5S$•0.5EtOAc) C, H, N.

(*R*)-*N*-(3-Methylbenzyl)-1,3-thiazolidine-4-carboxamide (18d). Compound **18d** was prepared from Boc-Thz-OH and 3-methylbenzylamine in a manner similar to that described for compound **18b**, yield 79%: mp 77−79 °C; ^{1}H NMR (DMSO-d_6) δ 2.28 (S, 3H), 2.91−2.97 (m, 3H), 3.28−3.38 (m, 1H), 3.85−3.93 (m, 1H), 4.10 (d, 2H, J = 9.7 Hz), 4.15−4.34 (m, 2H), 7.01−7.12 (br, 3H), 7.17−7.22 (m, 1H), 8.51 (br, 1H); MS (TOF) m/z = 237 (M^+ + H). Anal. ($C_{12}H_{16}N_2OS$) C, H, N.

(*R*)-*N*-(3-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (19d). Compound **19d** was prepared from Boc-Apns-OH and compound **18d** in a manner similar to that described for compound **19b**, yield 68%: mp 145−147 °C; ^{1}H NMR (DMSO-d_6) δ 1.4−1.6 (br, 2H), 2.19 (s, 3H), 2.2−2.4 (m, 1H), 2.7−2.9 (m, 1H), 3.1−3.4 (m, 3H), 4.05−4.22 (m, 3H), 4.76 (d, 1H, J = 8.6 Hz), 4.82 (d, 1H, J = 8.6 Hz), 4.95−4.98 (m, 1H), 5.36 (d, 1H, J = 7.8 Hz), 6.89−7.31 (m, 9H), 8.96 (t, 1H, J = 5.4 Hz); MS (TOF) m/z = 414 (M^+ + H). Anal. ($C_{22}H_{27}N_3O_3S$) C, H, N.

(*R*)-*N*-(3-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (21d). Compound **21d** was prepared from 3-acetoxy-2-methylbenzoic acid and compound **19d** in a manner similar to that described for compound **9c**, yield 40%: mp 112−117 °C; ^{1}H NMR (DMSO-d_6) δ 1.83 (s, 3H), 2.25 (s, 3H), 2.7−2.9 (m, 2H), 3.0−3.2 (m, 1H), 3.3−3.5 (m, 1H), 4.0−4.5 (m, 4H), 4.78−4.87 (m, 2H), 5.05 (d, 1H, J = 8.9 Hz), 5.46 (br, 1H), 6.56 (d, 1H, J = 7.3 Hz), 6.78 (d, 1H, J = 7.3 Hz), 6.92−7.34 (m, 10H), 8.13 (d, 1H, J = 8.4 Hz), 8.41 (br, 1H), 9.39 (s, 1H); MS (TOF) m/z = 548 (M^+ + H). Anal. ($C_{30}H_{33}N_3O_5S$•0.5EtOAc) C, H, N.

(*R*)-*N*-(4-Methylbenzyl)-1,3-thiazolidine-4-carboxamide (18e). Compound **18e** was prepared from Boc-Thz-OH and 4-methylbenzylamine in a manner similar to that described for compound **18b**, yield 85%: mp 122−124 °C; ^{1}H NMR

(DMSO-d_6) δ 2.27 (s, 3H), 2.95 (d, 2H, J = 6.5 Hz), 3.26−3.37 (m, 1H), 3.83−3.91 (m, 1H), 4.09 (d, 2H, J = 9.7 Hz), 4.17−4.32 (m, 3H), 7.12 (s, 4H), 8.49 (br, 1H); MS (TOF) m/z = 237 (M^+ + H). Anal. ($C_{12}H_{16}N_2OS$) C, H, N.

(*R*)-*N*-(4-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (19e). Compound **19e** was prepared from Boc-Apns-OH and compound **18e** in a manner similar to that described for compound **19b**, yield 58%: mp 153−155 °C; ^{1}H NMR (DMSO-d_6) δ 1.5−1.7 (br, 2H), 2.21 (s, 3H), 2.26−2.38 (m, 1H), 2.79 (t, 1H, J = 8.4 Hz), 3.1−3.4 (m, 3H), 4.07−4.20 (m, 3H), 4.75 (d, 1H, J = 8.4 Hz), 4.82 (d, 1H, J = 8.4 Hz), 4.93−4.97 (m, 1H), 5.37 (d, 1H, J = 7.8 Hz), 6.97−7.32 (m, 9H), 8.95 (t, 1H, J = 5.5 Hz); MS (TOF) m/z = 414 (M^+ + H). Anal. ($C_{22}H_{27}N_3O_3S$•0.5H_2O) C, H, N.

(*R*)-*N*-(4-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (21e). Compound **21e** was prepared from 3-acetoxy-2-methylbenzoic acid and compound **19e** in a manner similar to that described for compound **9c**, yield 57%: mp 113−116 °C; ^{1}H NMR (DMSO-d_6) δ 1.83 (s, 3H), 2.24 (s, 3H), 2.7−2.9 (m, 2H), 3.0−3.2 (m, 1H), 3.3−3.4 (m, 1H), 4.28−4.48 (m, 4H), 5.05 (d, 1H, J = 9.2 Hz), 5.48 (d, 1H, J = 6.2 Hz), 6.56 (d, 1H, J = 7.0 Hz), 6.78 (d, 1H, J = 7.8 Hz), 6.95 (t, 1H, J = 7.8 Hz), 7.06−7.34 (m, 9H), 8.13 (d, 1H, J = 7.8 Hz), 8.39 (br, 1H), 9.39 (s, 1H); MS (TOF) m/z = 548 (M^+ + H). Anal. ($C_{30}H_{33}N_3O_5S$•0.5EtOAc) C, H, N.

(*R*)-*N*-(2-Methylbenzyl)-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (18f). To a solution of Boc-Dmt-OH (5.22 g, 20.0 mmol) and triethylamine (3.06 mL, 22.0 mmol) in ethyl acetate (50 mL) was added DPP-Cl (4.55 mL, 22.0 mmol) in an ice bath, and the mixture was stirred for 1 h. Then to the reaction mixture were added 2-methylbenzylamine (2.73 mL, 22.0 mmol) and triethylamine (3.06 mL, 22.0 mmol) in an ice bath, and the mixture was stirred overnight. The reaction mixture was washed sequentially with 1 N HCl, 3% K_2CO_3, and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was redissolved in CH_2Cl_2 (30 mL), added to 4 N HCl in dioxane (30 mL), and stirred for 2 h. To the reaction mixture was added water, and aqueous phase was washed with toluene, neutralized with 2 N NaOH, and extracted with ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a crude product. Recrystallization from *n*-hexane/ethyl acetate gave 3.75 g of the title compound, yield 71%: mp 77−79 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (s, 3H), 1.52 (s, 3H), 2.28 (s, 3H), 3.27 (s, 3H), 3.66 (s, 1H), 4.03 (d, 1H, J = 9.6 Hz), 4.22−4.33 (m, 3H), 7.12−7.22 (m, 4H), 8.32−8.33 (br, 1H); MS (TOF) m/z = 265 (M^+ + H). Anal. ($C_{14}H_{20}N_2OS$) C, H, N.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (19f). To a solution of compound **18f** (15.0 g, 56.8 mmol) were added Boc-Apns-OH (16.0 g, 54.1 mmol), HOBt (7.30 g, 54.1 mmol) in DMF (150 mL), and EDC•HCl (11.4 g, 59.5 mmol) in an ice bath, and the mixture was stirred overnight. To the reaction mixture was added ethyl acetate, and the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was redissolved in CH_2Cl_2 (100 mL), added to 4 N HCl in dioxane (100 mL), and stirred for 3 h. The reaction mixture was concentrated under reduced pressure and then redissolved in water. The aqueous phase was washed with toluene, neutralized with 2 N NaOH, and gave the precipitate. The crude product was recrystallized from ethyl acetate and gave 11.0 g of the title compound, yield 46%: mp 205−208 °C; ^{1}H NMR (DMSO-d_6) δ 1.15−1.25 (br, 2H), 1.34 (s, 3H), 1.52 (s, 3H), 2.23−2.31 (m, 1H), 2.70 (t, 1H, J = 10.0 Hz), 3.09 (d, 1H, J = 12.3 Hz), 4.04−4.08 (br, 1H), 4.11 (dd, 1H, J = 5.0 Hz, 15.3 Hz), 4.22 (dd, 1H, J = 5.0 Hz, 15.0 Hz), 4.36 (s, 1H), 4.90 (s, 2H), 5.31 (d, 1H, J = 6.6 Hz), 6.95 (s, 3H), 7.12−7.31 (m, 6H), 8.48 (t, 1H, J = 4.8 Hz); MS (TOF) m/z = 442 (M^+ + H). Anal. ($C_{24}H_{31}N_3O_3S$) C, H, N.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (21f). To a solution of 3-acetoxy-2-methylbenzoic acid (4.27 g, 22.0 mmol) and triethylamine (3.06 mL, 22.0 mmol) in ethyl acetate (100 mL) was added DPP-Cl (4.55 mL, 22.0 mmol) in an ice bath, and the mixture was stirred for 1 h. Then to the reaction mixture were added compound **19f** (8.82 g, 20.0 mmol) and triethylamine (3.34 mL, 24.0 mmol) in an ice bath, and the mixture was stirred overnight. The reaction mixture was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine and concentrated. To the solution of the resulting residue in methanol (50 mL) was added 1 N NaOH (22 mL, 22.0 mmol), and the mixture was stirred for 1 h. The mixture was acidified with 1 N HCl, and the aqueous phase was extracted with ethyl acetate. The organic extract was washed sequentially with 1 N HCl, 3% K_2CO_3, and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was recrystallized from *n*-hexane/ethyl acetate and gave 9.70 g of the title compound, yield 84%: mp 137−139 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.83 (s, 3H, benzoyl-CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.7−2.9 (m, 2H, Apns-4-CH_2), 4.10 (dd, 1H, J = 5.0 Hz, 15.0 Hz, benzylamine-CH_2), 4.3−4.5 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.01 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.14 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.46 (d, 1H, J = 6.6 Hz, Apns-2-OH), 6.55 (d, 1H, J = 7.5 Hz, aromatic), 6.77 (d, 1H, J = 7.5 Hz, aromatic), 6.94 (t, 1H, J = 7.5 Hz, aromatic), 7.0−7.4 (m, 9H, aromatic), 8.13 (d, 1H, J = 8.7 Hz, Apns-NH), 8.31 (t, 1H, benzylamine-NH), 9.36 (s, 1H, benzoyl-OH); ^{13}C NMR (DMSO-d_6) δ 12.23, 18.67, 24.57, 30.27, 33.72, 40.42, 47.73, 51.08, 52.83, 71.50, 72.06, 115.14, 117.45, 121.41, 125.61, 125.68, 125.80, 126.87, 127.62, 127.82, 127.86, 128.01, 129.52, 129.78, 135.64, 136.73, 139.03, 139.30, 155.30, 167.96, 169.07, 170.12; MS (TOF) m/z = 576 (M^+ + H). Anal. ($C_{32}H_{37}N_3O_5S$) C, H, N.

(*R*)-*N*-(2-Chlorobenzyl)-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (18g). Compound **18g** was prepared from Boc-Dmt-OH and 2-chlorobenzylamine in a manner similar to that described for compound **18f**, yield 46%: mp 93−96 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (s, 3H), 1.54 (s, 3H), 3.28−3.34 (m, 1H), 3.6−3.8 (br, 1H), 4.05 (t, 1H, J = 10.3 Hz), 4.26−4.49 (m, 3H), 7.27−7.47 (m, 4H), 8.51 (br, 1H); MS (TOF) m/z = 285 (M^+ + H). Anal. ($C_{13}H_{17}ClN_2OS$) C, H, N.

(*R*)-*N*-(2-Chlorobenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (19g). Compound **19g** was prepared from Boc-Apns-OH and compound **18g** in a manner similar to that described for compound **19f**, yield 59%: mp 175−178 °C; ^{1}H NMR (DMSO-d_6) δ 1.33 (s, 3H), 1.4−1.5 (m, 2H), 1.53 (s, 3H), 2.31−2.39 (m, 1H), 2.79−2.87 (m, 1H), 3.08−3.14 (m, 1H), 4.11 (t, 1H, J = 7.7 Hz), 4.26−4.36 (m, 2H), 4.39 (s, 1H), 4.95 (s, 2H), 5.38 (d, 1H, J = 7.8 Hz), 7.17−7.39 (m, 9H), 8.85 (t, 1H, J = 5.7 Hz); MS (TOF) m/z = 462 (M^+ + H). Anal. ($C_{23}H_{28}ClN_3O_3S$) C, H, N.

(*R*)-*N*-(2-Chlorobenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (21g). Compound **21h** was prepared from 3-acetoxy-2-methylbenzoic acid and compound **19g** in a manner similar to that described for compound **21f**, yield 82%: mp 147−149 °C; ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 3H), 1.52 (s, 3H), 1.83 (s, 3H), 2.6−2.9 (m, 2H), 4.2−4.4 (m, 1H), 4.4−4.6 (m, 4H), 5.03 (d, 1H, J = 9.2 Hz), 5.16 (d, 1H, J = 8.9 Hz), 5.51 (d, 1H, J = 6.8 Hz), 6.55 (d, 1H, J = 7.0 Hz), 6.78 (d, 1H, J = 7.3 Hz), 6.95 (t, 1H, J = 7.6 Hz), 7.1−7.3 (m, 7H), 7.39−7.50 (m, 2H), 8.14 (d, 1H, J = 8.4 Hz), 8.52 (br, 1H), 9.38 (s, 1H); MS (TOF) m/z = 596 (M^+ + H). Anal. ($C_{31}H_{34}ClN_3O_5S$•0.5EtOAc) C, H, N.

(*R*)-*N*-(2-Trifluoromethylbenzyl)-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (18h). Compound **18h** was prepared from Boc-Dmt-OH and 2-trifluoromethylbenzylamine in a manner similar to that described for compound **18f**, yield 36%: mp 118−120 °C; ^{1}H NMR (DMSO-d_6) δ 1.17 (s, 3H), 1.55 (s, 3H), 3.31−3.36 (m, 1H), 3.69−3.78 (m, 1H), 4.03−4.10 (m, 1H), 4.29 (t, 1H, J = 8.0 Hz), 4.50 (t, 2H, J = 5.1 Hz), 7.46−7.55 (m, 2H), 7.65−7.74 (m, 2H), 8.56 (t, 1H, J = 5.5 Hz); MS (TOF) m/z = 319 (M^+ + H). Anal. ($C_{14}H_{17}F_3N_2OS$) C, H, N.

(*R*)-*N*-(2-Trifluoromethylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (19h). Compound **19h** was prepared from Boc-Apns-OH and compound **18h** in a manner similar to that described for compound **19f**, yield 55%: mp 192−195 °C; ^{1}H NMR (DMSO-d_6) δ 1.34 (s, 3H), 1.4−1.5 (m, 2H), 1.54 (s, 3H), 2.31−2.39 (m, 1H), 2.79−2.85 (m, 1H), 3.07−3.33 (m, 1H), 4.12 (t, 1H, J = 8.1 Hz), 4.40 (s, 3H), 4.96 (s, 2H), 5.40 (d, 1H, J = 8.4 Hz), 7.16−7.39 (m, 6H), 7.49−7.63 (m, 3H), 8.90 (t, 1H, J = 5.5 Hz); MS (TOF) m/z = 496 (M^+ + H). Anal. ($C_{24}H_{28}F_3N_3O_3S$) C, H, N.

(*R*)-*N*-(2-Trifluoromethylbenzyl)-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (21h). Compound **21h** was prepared from 3-acetoxy-2-methylbenzoic acid and compound **19h** in a manner similar to that described for compound **21f**, yield 89%: mp 142−145 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 3H), 1.54 (s, 3H), 1.82 (s, 3H), 2.65−2.85 (m, 2H), 4.3−4.7 (m, 5H), 5.04 (d, 1H, J = 8.9 Hz), 5.20 (d, 1H, J = 8.9 Hz), 5.51 (d, 1H, J = 7.0 Hz), 6.55 (d, 1H, J = 7.3 Hz), 6.78 (d, 1H, J = 8.4 Hz), 6.94 (t, 1H, J = 7.8 Hz), 7.13−7.27 (m, 5H), 7.40 (t, 1H, J = 7.7 Hz), 7.55 (t, 1H, J = 7.6 Hz), 7.68 (d, 1H, J = 7.8 Hz), 8.18 (d, 1H, J = 8.4 Hz), 8.63 (t, 1H, J = 5.5 Hz), 9.38 (s, 1H); MS (TOF) m/z = 630 (M^+ + H). Anal. ($C_{32}H_{34}F_3N_3O_5S$) C, H, N.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-(2-ethyl-3-hydroxybenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (21i). Compound **21i** was prepared from 3-acetoxy-2-ethylbenzoic acid and compound **19f** in a manner similar to that described for compound **21f**, yield 85%: mp 164−167 °C; ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H, J = 7.4 Hz), 1.36 (s, 3H), 1.50 (s, 3H), 2.26 (s, 3H), 2.2−2.4 (m, 2H), 2.7−2.9 (m, 2H), 4.05−4.20 (m, 1H), 4.37−4.50 (m, 4H), 5.01 (d, 1H, J = 9.2 Hz), 5.15 (d, 1H, J = 9.5 Hz), 5.46 (d, 1H, J = 6.8 Hz), 6.55 (d, 1H, J = 6.8 Hz), 6.78 (d, 1H, J = 7.3 Hz), 6.95 (t, 1H, J = 7.6 Hz), 7.09−7.35 (m, 9H), 8.14 (d, 1H, J = 8.1 Hz), 8.33 (br, 1H), 9.34 (s, 1H); MS (TOF) m/z = 590 (M^+ + H). Anal. ($C_{33}H_{39}N_3O_5S$) C, H, N.

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