Combination of Non-natural D-Amino Acid Derivatives and Allophenylnorstatine−Dimethylthioproline Scaffold in HIV Protease Inhibitors Have High Efficacy in Mutant HIV

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Received December 12, 2007

Several non-natural D-amino acid derivatives were introduced as P_2/P_3 residues in allophenylnorstatine-containing (Apns; (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid) HIV protease inhibitors. The synthetic analogues exhibited potent inhibitory activity against HIV-1 protease enzyme and HIV-1 replication in MT-4 cells. Structure–activity relationships revealed that D-cysteine or serine derivatives contributed to highly potent anti-HIV activities. Interestingly, anti-HIV activity of all the D-amino acid-introduced inhibitors was remarkably enhanced in their anti-HIV activities against a Nelfinavir-resistant clone, which has a D30N mutation in the protease, over that of the wild-type strain. HIV inhibitory activity of several analogues was moderately affected by an inclusion of α_1-acid glycoprotein in the test medium.

Introduction

Continuous pandemic of human immunodeficiency virus (HIV[a]) infection, particularly a recent striking increase in Asia, Eastern Europe, and Africa, is still recognized as a serious problem that causes acquired immune deficiency syndrome (AIDS) and increases mortality.[1] To treat HIV infection, therapies involving more than two drugs from reverse transcriptase inhibitors, protease inhibitors, and a fusion inhibitor, called HAART (highly active antiretroviral therapy), form an effective options to reduce plasma HIV mRNA to undetectable level.[2] Despite the success of such chemotherapy, treatment is life-long while monitoring that HIV resistance to these clinical drugs do not develop. HIV type-1 (HIV-1) protease is a homodimeric aspartic protease that processes its own polyproteins, Pr55 *gag* and Pr160 *gag-pol*, which contain all the structural and catalytic proteins required for the formation of mature and infectious virions.[3] Mutations in the HIV-1 protease region is also known to cause resistance to clinically used HIV protease inhibitors.[4] Tipranavir[5] and Darunavir[6] have recently been approved by the FDA for use in salvage therapies against the emergence of HIV mutants that are resistant to available drugs. Multimutations via natural polymorphism in other HIV subtypes, however, could develop corresponding resistances in the future.[7,8] Consequently, the development of structurally new and highly potent HIV protease inhibitors with different resistance profiles is still of interest.[9,10]

We have previously described the design and synthesis of highly potent peptidomimetic HIV protease inhibitors, **1** (KNI-272[11,12]) and **2** (KNI-764,[13–15] also known as JE-2147, AG-1776, SM-319777), which contain allophenylnorstatine (Apns; (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid) with a hydroxymethylcarbonyl (HMC) isostere as a transition-state mimic as the P_1 residue.[16–18] Considering that HIV-1 protease prefers for the cleavage site specific sequences Phe-Pro and Tyr-Pro, (*R*)-1,3-thiazolidine-4-carboxylic acid (Thz) and (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (Dmt) serve as alternatives for the P_1' proline residue (Figure 1). X-ray and NMR analyses of an Apns-containing inhibitor−protease complex revealed hydrogen bond interactions between the HMC and the two catalytic Asps, that is, HMC is an ideal transition-state mimic.[11a,19,20] The concept resulted in the development of **1** and **2** as clinical candidates. On the basis of the structure–activity relationships of HIV-1 protease inhibitors, we identified a preliminary series of compounds[21,22] that exhibited extremely potent enzyme-inhibitory activity, lower cytotoxicity, and high antiviral activities against both wild-type and mutant proteases. In the present study, we describe our continuing efforts to generate novel HIV protease inhibitors containing various P_2/P_3 non-natural D-amino acid analogues, leading to highly potent compounds whose potencies surpass those of clinically used drugs against wild-type and drug-resistant HIVs, while not being significantly affected by undesired protein binding. Of the 32 compounds described in this present work, 12 preliminary compounds that have been briefly reported[22] are elaborated in full detail in relation to a much larger data sample size so as to obtain a more accurate and deeper interpretation of structure–activity relationships. Moreover, specific binding to serum protein, particularly α_1-acid glycoprotein (AGP), is known to drastically reduce the potency of clinically used drugs. Concerned by the detrimental effect of AGP-binding, in the current work, we have carried out the evaluation of some interesting compounds in the presence and absence of AGP.

Design Rationale. Information on the interactions between compound **1** and HIV-1 protease based on X-ray crystallographic studies of the complex[19] and molecular modeling[23]

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[a] Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; HAART, highly active antiretroviral therapy; Apns, allophenylnorstatine; HMC, hydroxymethylcarbonyl; Thz, (*R*)-1,3-thiazolidine-4-carboxylic acid; Dmt, (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid; AGP, α_1-acid glycoprotein; Boc, *tert*-butyloxycarbonyl; Hph, homophenylalanine; EDC, 1-ethyl-3-(3,3-dimethylamino-propyl)carbodiimide; HOBt, 1-hydroxybenzotriazole; RTV, Ritonavir; NFV, Nelfinavir; rmsd, root-mean-square deviations; SI, selectivity index; MD, molecular dynamics.

10.1021/jm701555p CCC: $40.75
Published on Web 04/22/2008

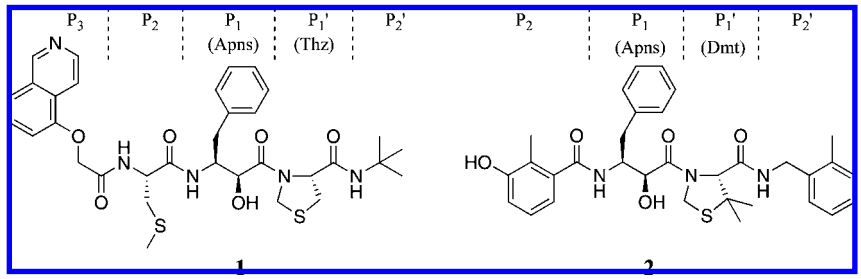


Figure 1. Structures of clinically tested Apns-containing HIV protease inhibitors.

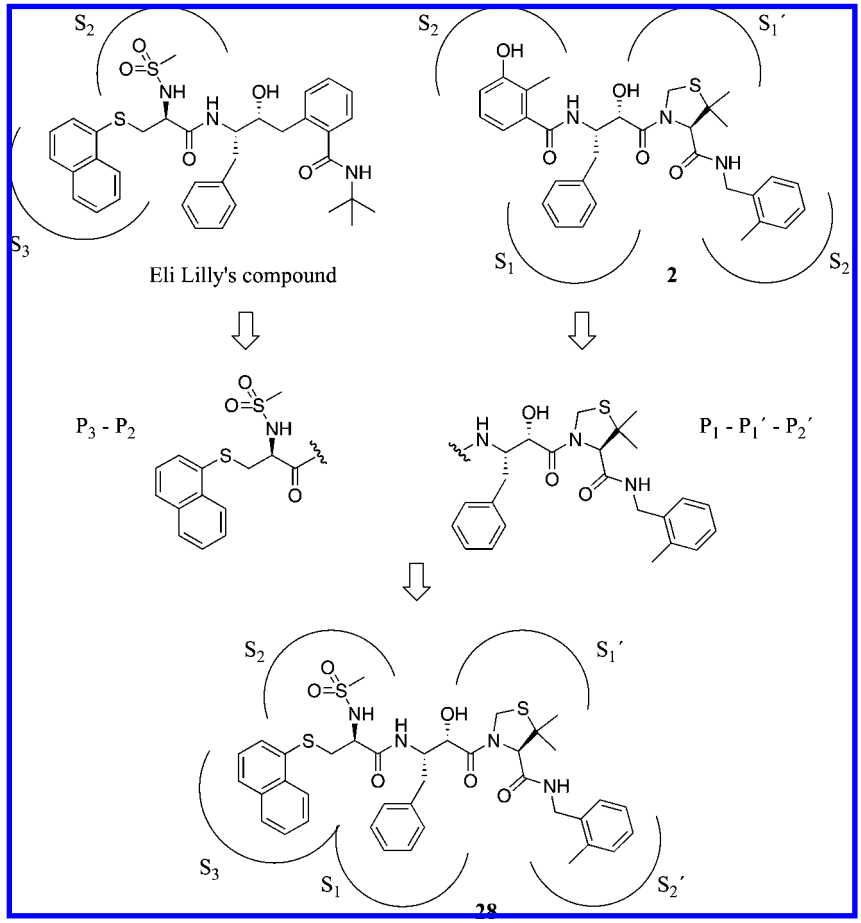
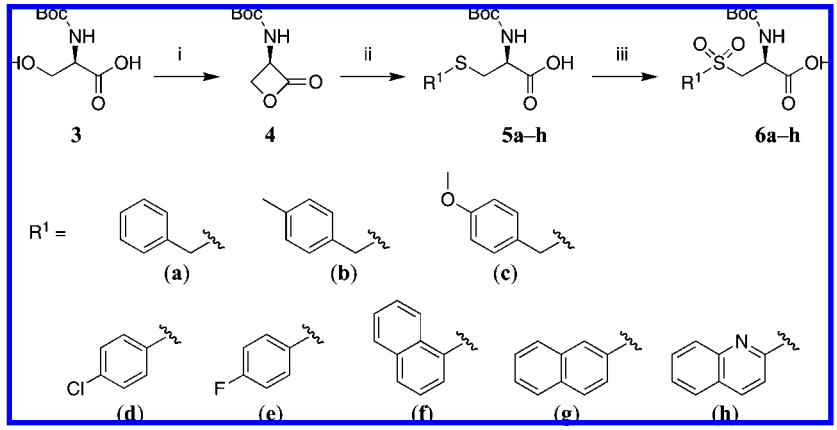


Figure 2. Incorporation of D-amino acid derivative into Apns-Dmt scaffold.

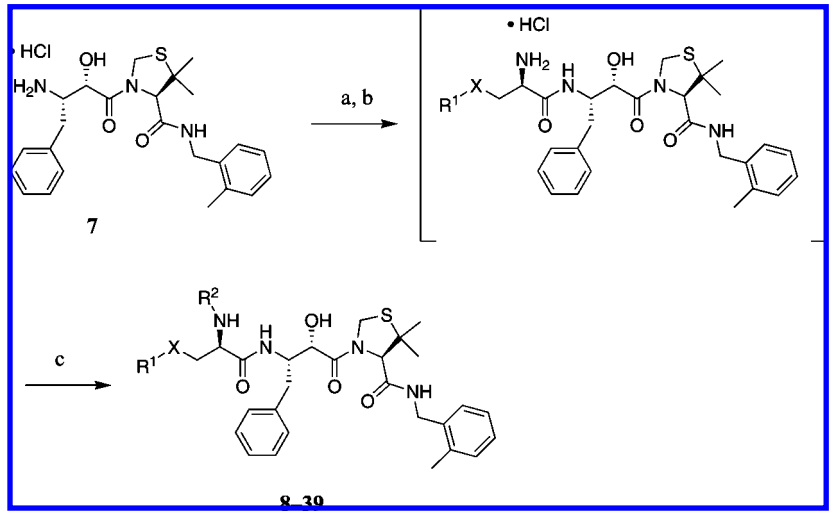
suggest a feasible replacement of the L-amino acid residue with different moieties at the P_2/P_3 positions to increase interactions with the enzyme. A P_2 methylthioalanine is not preferred according to a pharmacokinetic study with **1**[12] because the sulfur atom is oxidized in physiological conditions, resulting in a short plasma half-life. In 1994, Eli Lilly group reported an incorporation of non-natural D-amino acids at the P_2/P_3 positions of HIV protease inhibitors with an hydroxyethylene isostere.[24] The D-amino acid residue was suggested to bind as the side chain points toward the S_3 pocket of the protease and the substituted α-amino group occupies the S_2 pocket (Figure 2). Eli Lilly group named this idea as the "D-amino acid concept" and optimized the non-natural D-amino acid residue to exhibit adequate antiviral potency for oral availability in rat.[25,26] Later, Fujii and coworkers reported an application of the D-amino acid concept to hydroxyethylamine isosteres.[27] Among their compounds, two sulfoxide analogues maintained their potencies against multidrug resistant HIV-1 strains. These reports prompted us to disclose our independent approach, which is the incorporation of various D-amino acid derivatives as the P_2/P_3 residues to the Apns-Dmt scaffold. D-Amino acid-containing inhibitors may sustain their plasma concentration longer than our previous lead compounds because of their stability. In our design, compound **2** was used as a template to replace its P_2 hydroxylmethylbenzoyl moiety with a D-amino acid residue. The amino group of the D-amino acid residue was substituted with a mesyl or acetyl group, which are effective to mimic the L-asparagine residue in the Eli Lilly's report. A virtual compound containing D-*N*-mesyl-*S*-naphthylcysteine (Figure 2) suggested a preferred binding orientation in the active site of HIV protease by a molecular modeling simulation as the *S*-naphthylthiomethyl side chain points to the S_3 pocket and the *N*-mesylamino group is oriented in the S_2 pocket. We diversified the side chain and *N*-substituent of the D-amino acid residue at three specific positions in order to optimize, within our Apns-Dmt scaffold, for enzyme–inhibitory and antiviral activity.

Chemistry. We synthesized D-cysteine analogues following a precedent study of Eli Lilly's group. The D-cysteine derivatives

Scheme 1. Reagents: (i) Ph_3P, DEAD, −78 °C (ii) R^1-SNa, THF; (iii) *m*-CPBA (2 equiv), CH_2Cl_2



Scheme 2. Reagents: (a) Boc-D-Hph-OH or Boc-D-Ser(Bzl)-OH or **5a**−**h** or **6a**−**h**, EDC, HOBt, Et_3N, DMF; (b) 4N HCl-dioxane; (c) Ac_2O or MsCl, NMM, CH_2Cl_2

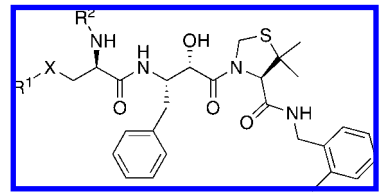


were prepared by a ring-opening reaction of Vederas' *N*-Boc-D-serine β-lactone[28] (**4**), starting from *N*-Boc-D-serine (**3**), with a variety of mercaptans in a single step (**5a**, **5b**, **5d**−**h**) (Scheme 1). For R^1 *p*-methoxybenzyl analogues, commercially available *N*-Boc-D-(*S*-*p*-methoxybenzyl)cysteine (**5c**) was used. Additional oxidation of D-*N*-Boc-cysteine analogues using *m*-chloroperbenzoic acid provided the corresponding sulfones (**6a**−**h**). The incorporation of D-amino acid derivatives is shown in Scheme 2. As a first attempt to apply the "D-amino acid concept" to our Apns-Dmt skeleton, we chose commercially available optically pure *N*-Boc protected D-homophenylalanine (Boc-D-Hph-OH). An amine intermediate **7**, H-Apns-Dmt-(2-methyl)benzylamide, was prepared as previously reported.[14] The D-amino acid was coupled with **7** using the conventional EDC-HOBt method to afford Boc-protected intermediate. After deprotection of the Boc group, the mesyl and acetyl groups were attached to the amino group to afford the *N*-substituted analogues **8** and **9**, respectively. We introduced another commercially available *N*-Boc protected D-*O*-benzylserine, leading to the corresponding *N*-substituted analogues **10**, **11** using the same procedure as described above. Each sulfoxide and sulfone was condensed with amine **7**, followed by the removal of Boc-group and the treatment of mesyl chloride or acetic anhydride to give compounds **12**–**39**.

Results and Discussion

The D-homophenylalanine analogue was the first attempt to confirm the function of the "D-amino acid concept" to our Apns-Dmt scaffold. Compounds **8** and **9**, which have a mesyl and an acetyl, respectively, possessed an R^1 benzyl without an X moiety. These compounds exhibited nanomolar HIV-1 protease inhibition (about 50% at 1 nM inhibitor, Table 1) with a slight difference between the R^2 groups. This result evidenced the compatibility of D-amino acid alteration to our scaffold. Incorporation of an oxygen atom at the X position in D-serine derivatives increased protease inhibitory activity (**10** and **11**). Compounds **12** and **13**, with a replacement of the oxygen to sulfur atom in the D-cysteine structure, exhibited improved potencies. In the case of O or S at the X position, the acetyl moiety was slightly better for inhibitory potency than the mesyl group. We also tested the oxidated cysteine derivative, i.e., sulfonyl (X = SO_2) analogues. The resultant mesyl form **14** was more potent than the acetyl form **15** with moderate activity. Among these R^1 benzyl analogues, a combination of an X sulfur with an R^2 acetyl (compound **13**) exhibited the best result for enzyme inhibition. Inhibitory activity against HIV-1 IIIB of these R^1 benzyl analogues (**8**–**15**) was evaluated in MT-4 cells. EC_{50} values ranged from 15 to 219 nM. The contribution of X structure to EC_{50} value seemed to be higher than that of enzyme-inhibitory activity. The anti-HIV activity of X sulfonyl derivatives **14** and **15** was dramatically attenuated compared to other X moieties with R^1 benzyl analogues. As a result, the combination of an X sulfur with an R^2 acetyl was preferred for anti-HIV activity as well as protease inhibition.

Table 1. HIV-1 Protease Inhibitory Activity and Anti-HIV-1 Activity



compound	structure			HIV-1 PR inhibition		anti-HIV activity (HIV-1IIIB) MT-4/MTT		
	R^1	X	R^2	% at 1 nM	IC_{50}, nM	EC_{50}, nM	CC_{50}, nM	SI
8 (KNI-1827)	benzyl		Ms	55		59	11000	190
9 (KNI-1880)	benzyl		Ac	45		51	>31000	>610
10 (KNI-1876)	benzyl	O	Ms	59	0.56	20	17000	850
11 (KNI-1878)	benzyl	O	Ac	64	0.39	21	>30000	>1400
12 (KNI-1831)	benzyl	S	Ms	66		17	9800	580
13 (KNI-1976)	benzyl	S	Ac	81		15	15000	1000
14 (KNI-1992)	benzyl	SO_2	Ms	66		219	>27000	>120
15 (KNI-1991)	benzyl	SO_2	Ac	47		117	>28000	>240
16 (KNI-1987)	*p*-methylbenzyl	S	Ms	58		17	8900	520
17 (KNI-1881)	*p*-methylbenzyl	S	Ac	76		12	8800	730
18 (KNI-2015)	*p*-methylbenzyl	SO_2	Ms	52		125	>26000	>210
19 (KNI-2016)	*p*-methylbenzyl	SO_2	Ac	53		82	>28000	>340
20 (KNI-1932)	*p*-methoxybenzyl	S	Ms	77	0.26	9.3	10000	1100
21 (KNI-1931)	*p*-methoxybenzyl	S	Ac	87	0.18	13	17000	1300
22 (KNI-1986)	*p*-methoxybenzyl	SO_2	Ms	66		203	>26000	>130
23 (KNI-1990)	*p*-methoxybenzyl	SO_2	Ac	59		102	>27000	>260
24 (KNI-1910)	*p*-chlorophenyl	SO_2	Ms	95	0.11	nt	nt	nt
25 (KNI-1909)	*p*-chlorophenyl	SO_2	Ac	80	0.22	47	18000	380
26 (KNI-1993)	*p*-fluorophenyl	SO_2	Ms	94		212	>27000	>130
27 (KNI-1960)	*p*-fluorophenyl	SO_2	Ac	79	0.27	105	>28000	>270
28 (KNI-1966)	1-naphthyl	S	Ms	74		6.4	4400	690
29 (KNI-1964)	1-naphthyl	S	Ac	68		17	8000	470
30 (KNI-1933)	1-naphthyl	SO_2	Ms	97	0.083	61	19000	310
31 (KNI-1961)	1-naphthyl	SO_2	Ac	97	0.090	40	16000	400
32 (KNI-1947)	2-naphthyl	S	Ms	67		13	6100	470
33 (KNI-1946)	2-naphthyl	S	Ac	53		18	8300	460
34 (KNI-1969)	2-naphthyl	SO_2	Ms	98	0.090	81	19000	230
35 (KNI-1965)	2-naphthyl	SO_2	Ac	96	0.086	28	16000	570
36 (KNI-1935)	2-quinolinyl	S	Ms	28		169	8500	50
37 (KNI-1934)	2-quinolinyl	S	Ac	27		151	9700	64
38 (KNI-1938)	2-quinolinyl	SO_2	Ms	91		473	>26000	>55
39 (KNI-1937)	2-quinolinyl	SO_2	Ac	85		94	>27000	>290
1				40		22	>30000	>1400
Ritonavir				90		36	17000	470
Nelfinavir				29		16	11000	690

We focused on the sulfur and sulfonyl for the X structure because of their relatively stronger enzyme-inhibitory activity and synthetic prospects. Derivatives **16**–**23** with a methyl or methoxy substitution at the *p*-position of the R^1 benzyl moiety were evaluated. The methoxy substituent was better for HIV protease inhibition than the methyl substituent in the case of the X sulfur (compounds **16**, **17** < compounds **20**, **21**). Anti-HIV activities of these compounds were similarly potent; compound **21** exhibited an EC_{50} value of 9.3 nM. On the other hand, compounds **18**, **19**, **22**, and **23** with an X sulfonyl moiety were less potent against not only the protease but also the virus.

Replacements of the R^1 benzyl group with *p*-chlorophenyl and *p*-fluorophenyl groups resulted in improved enzyme inhibition (**24**–**27**), especially compounds **24** and **26**, with a R^2 mesyl group exhibiting extremely potent enzyme inhibition (>90% inhibition at 1nM). The anti-HIV activities of **25**–**27** were as low as the R^1 benzyl derivatives with an X SO_2 moiety because of the disadvantage of the sulfonyl group observed in the first modification. Compound **25** with an R^1 *p*-chlorophenyl and a R^2 acetyl was relatively potent (47 nM) against the virus among the X sulfonyl derivatives.

These results from the R^1 halophenyl derivatives encouraged us to introduce planar moieties such as a naphthyl group. Compounds **28**–**35** possess 1-naphthyl or 2-naphthyl as R^1. In this series, it is clear that compounds with an X sulfonyl were more potent than that of a sulfur in enzyme inhibition (**30**, **31**, **34**, and **35** with 96–98%). The IC_{50} values of these sulfonyl moiety ranged from 80 to 90 pM. Unfortunately, we observed a decrease in anti-HIV activity, which is similar to that observed with our previous compounds, that is, an X sulfur was better than a sulfonyl in antiviral assay. The difference between the R^2 mesyl and acetyl was ambiguous against enzyme inhibition but rather obvious against antiviral activity. Compound **28** was the most potent against HIV in the D-amino acid series with an EC_{50} value of 6.4 nM, exceeding the potency of Ritonavir (RTV) and Nelfinavir (NFV) despite its moderate potency against the protease. R^1 quinolinyl derivatives, with a minute replacement of a nitrogen atom in the 2-naphthyl group, dramatically attenuated potency (**36**–**39**). The decreased activity of these compounds indicated that an improper substitution of a polar nitrogen atom disrupted the hydrophobic interactions with the enzyme as evidenced by a 2-naphthyl group. Equation 1 shows

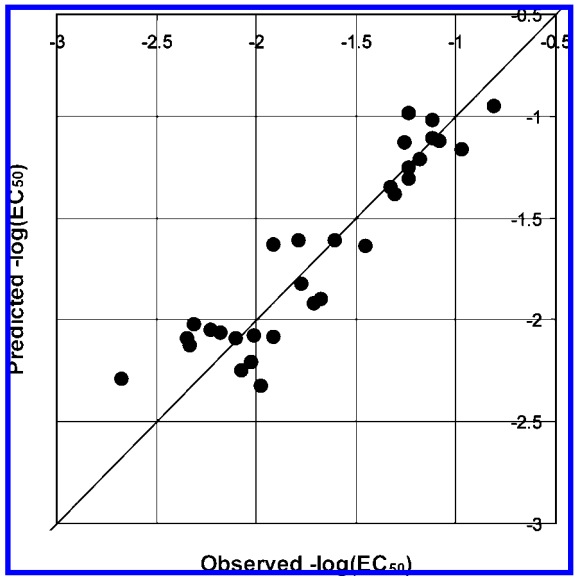


Figure 3. Chart showing agreement between predicted and observed anti-HIV activity: log(EC_{50}) values for compounds **8**–**23** and **25**–**39**. Predicted values were calculated from eq 1.

a quantitative structure–activity relationship correlating HIV protease inhibition and structural features with cellular antiviral EC_{50}.

$$-\log(EC_{50}) = 0.597\,(\text{Enz}) + 0.649\,(R_1) + 0.789\,(\text{X}) - 2.855 \quad n = 31,\ r_2 = 0.86,\ F = 56,\ p < 0.001 \quad (1)$$

where Enz: normalized log (% HIV protease inhibition at 1 nM), ranging from 0 to 1; R^1: benzyl = 0.531, *p*-methoxybenzyl = 0.707, *p*-methylbenzyl = 0.639, *p*-chlorophenyl = 0.693, *p*-fluorophenyl = 0.226, 1-naphthyl = 1.000, 2-naphthyl = 0.963, 2-quinolinyl = 0.000, ranging from 0 to 1; X: null = 0.450, O = 0.964, S = 1.000, SO_2 = 0.000, ranging from 0 to 1.

In our preliminary report, we derived a well-fitted, normalized, multiple collinear, quantitative SAR equation in which HIV protease inhibition and the nature of the X moiety were parametrized to correlate with antiviral activity (EC_{50}).[22] The R^1 and R^2 moieties were excluded as descriptors because they did not greatly contribute to the overall equation. Although, at the time, we only considered R^1 as *p*-methoxybenzyl, 1-naphthyl, and 2-naphthyl moieties (**20**–**23** and **28**–**35**), the current series of compounds has a larger complexity of R^1 moieties to such an extent that the R^1 has become involved as a descriptor (eq 1). Each descriptor, namely Enz, R^1, and X, is a normalized percentile value from 0 to 1, where 1 is the most potent and 0 is the least potent based on EC_{50} values. To be exact, to award scores to the R^1 and X descriptors, groups of compounds, where only one portion of the structure was changed (either R^1, X, or R^2), were ranked as normalized percentiles from 0 to 1 based on their EC_{50} values. To compare between the groups, the average of the respective normalized percentiles represented the score of the moiety being examined. The scores suggest that compounds containing an R^1 1-naphthyl or 2-naphthyl moiety and an X oxygen or sulfur atom are expected to exhibit potent antiviral activity. On the other hand, compounds possessing an R^1 2-quinolinyl or *p*-fluorophenyl moiety and an X SO_2 moiety are expected to exhibit low antiviral activity. The final equation is well fitted ($r^2 = 0.86$, Figure 3) and statistically significant ($p < 0.001$). A form of K-fold cross-validation, namely leave-one-out cross validation, was performed using a single observation from the original sample as the validation data and the remaining observations as the training data, and the process was repeated such that each observation in the sample was used once as the validation data. The equation is valid because the root-mean-square deviations (rmsd) of the coefficients and intercept are relatively small (0.597 ± 0.055, 0.649 ± 0.053, 0.789 ± 0.017, −2.855 ± 0.035), and the coefficient of determination did not greatly vary during cross-validation ($r^2 = 0.85-0.88$). Overall, each descriptor contributed almost equally to the equation (Enz, 29%; R^1, 32%; X, 39%).

The cytotoxicity of these D-amino acid series were sufficiently low and shown at the μM level (Table 1). Consequently, the selectivity index (SI) values of the D-amino acid derivatives were high. This result elucidated that our selective inhibitor design with an Apns-Dmt scaffold is based on the Phe-Pro residues of the virus-specific cleavage substrate. Exceptions are analogues with 2-quinolinyl structures, possessing relatively high toxicity. Concerning high anti-HIV activity and SI value (>1000), compounds **11**, **13**, **20**, and **21** would be potential candidates for further pharmacokinetic study.

RTV and NFV are widely used in clinical therapy. Recently, RTV has been used only for boosting as a CYP3A4 inhibitor to maintain plasma levels of other protease inhibitors.[2] The potency of RTV and NFV is reduced in plasma because of specific binding to serum protein,[29] especially AGP. The plasma level of AGP is reported to be increased in some HIV infected patients,[30] suggesting that a low plasma concentration of the free drugs would bind the protease and, therefore, the resistance of HIV to these drugs may easily occur. We selected some compounds with interesting potencies against wild-type HIV-1 to test against drug resistant mutants. The susceptibility of these D-amino acid derivatives against HIV-1 molecular clones of the NL-432 strain, which have resistance to each or both of RTV and NFV, is summarized in Table 2. The D-amino acid-containing compounds exhibited fairly potent activities against the wild-type NL-432 strain as tested to HIV-1 IIIB. The RTV-resistant clone is 2.8-fold less sensitive to RTV, while NFV maintained its activity (1.1-fold). Compound **1** distinctly attenuated its activity against the RTV-resistant strain (5.6-fold). On the other hand, compounds with D-amino acid derivatives generally exhibited some reduction as much as 4-fold in anti-HIV activity smaller than that of **1**. The most potent compound against RTV-resistant strain was **28** with an EC_{50} value of 17 nM, reducing its activity 1.8-fold from the wild-type strain. Among the D-amino acid-containing series, compound **21** maintained its activity (1.1-fold). On the other hand, an increase in potency was observed in the case of compound **25** with a relatively low activity. At the three mutation sites, N37S and G57R are polymorphisms derived from the wild-type protease of HIV-1 IIIB, therefore I84V is a critical change in the enzyme binding sites. These results suggest that the binding of the D-amino acid moiety is less dependent on the I84V mutation on the protease than the binding of the P_2/P_3 residue in **1**.

NFV drastically attenuated its anti-HIV activity (12-fold) against the NFV-resistant clone, while RTV was more sensitive (0.20-fold). The D30N mutation in the protease of the resistant strain is one of the main reasons for the decrease because a hydrogen bond interaction between NFV and Asp30 of the protease, observed in the crystal structure, may not be maintained in the Asn30 mutation. Compound **1** also diminished its potency against NFV-resistant clone (2.3-fold), although all of the D-amino acid derivatives surprisingly exhibited higher activity against the mutant than wild-type HIV (from 0.04- to 0.42-fold). These results proposed improvements in hydrophobic

Table 2. Inhibitory Activity against Drug-Resistant HIV-1 Strains

	structure			EC_{50}, nM (fold resistance)					
compound	R^1	X	R^2	WT (NL-432)	RTV resistant[a]		NFV resistant[b]		RTV and NFV resistant[c]	
9	benzyl		Ac	78	101	(1.3)	18	(0.23)	132	(1.7)
10	benzyl	O	Ms	22	90	(4.1)	2.3	(0.10)	92	(4.2)
11	benzyl	O	Ac	23	77	(3.3)	6.5	(0.28)	44	(1.9)
12	benzyl	S	Ms	20	63	(3.2)	1.8	(0.09)	55	(2.8)
13	benzyl	S	Ac	18	40	(2.2)	5.9	(0.33)	21	(1.2)
16	*p*-methylbenzyl	S	Ms	17	58	(3.4)	1.5	(0.09)	43	(2.5)
17	*p*-methylbenzyl	S	Ac	22	41	(1.9)	3.6	(0.16)	27	(1.2)
20	*p*-methoxybenzyl	S	Ms	16	20	(1.3)	0.67	(0.04)	23	(1.4)
21	*p*-methoxybenzyl	S	Ac	17	18	(1.1)	2.4	(0.14)	14	(0.82)
25	*p*-chlorophenyl	SO_2	Ac	88	44	(0.50)	9.6	(0.11)	19	(0.22)
28	1-naphthyl	S	Ms	9.3	17	(1.8)	0.67	(0.07)	13	(1.4)
29	1-naphthyl	S	Ac	20	70	(3.5)	6.3	(0.32)	52	(2.6)
32	2-naphthyl	S	Ms	17	31	(1.8)	0.80	(0.05)	23	(1.4)
33	2-naphthyl	S	Ac	17	65	(3.8)	7.2	(0.42)	32	(1.9)
1				36	201	(5.6)	84	(2.3)	237	(6.6)
Ritonavir				80	220	(2.8)	16	(0.20)	162	(2.0)
Nelfinavir				25	28	(1.1)	308	(12)	67	(2.7)

[a] Ritonavir-resistant clone from NL-432 strain established by Shionogi Co. Ltd.: N37S, G57R, and I84V. [b] Nelfinavir-resistant clone from NL-432 strain established by Shionogi Co. Ltd.: D30N, N37S, and G57R. [c] Ritonavir- and Nelfinavir-resistant clone from NL-432 strain established by Shionogi Co. Ltd.: L19V, V32I, M46L, G57R, L63P, and I85V.

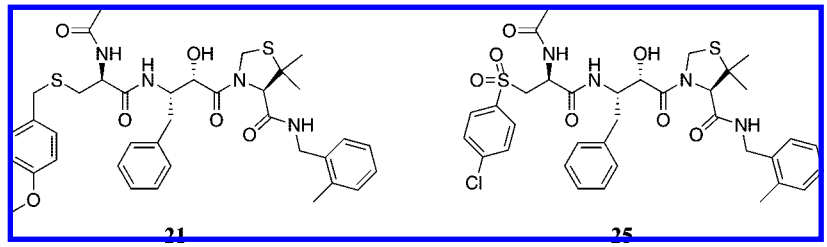


Figure 4. Structures of compounds **21** and **25**.

interactions of the D-amino acid moiety with Asn30 of the protease similar to that of RTV.

The RTV/NFV-resistant clone was less sensitive to both RTV and NFV, with 2.0- and 2.7-fold decreases, respectively. This clone possesses multiple mutations of L19V, V32I, S37N, M46L, L63P, and I85V compared to the original protease sequence of HIV-1 IIIB containing N37S and G57R. Among these mutations, V32I is thought to directly change the shape of the protease pockets. The activity of **1** was reduced by 6.6-fold. The results of the D-amino acid containing analogues against the RTV/NFV-resistant clone were similar to the results against the RTV-resistant clone. Interestingly, compound **21** maintained its activity (0.82-fold), similar to the RTV-resistant clone. In the series, compound **28** was the most potent with a decreased activity (1.4-fold). On the other hand, the activity of compound **25** was increased to a similar extent as the compound's potency against the RTV-resistant HIV strain. Compound **25** exhibited higher potencies against the three tested mutant proteases than the wild type protease. We believe that because the active sites of the mutant proteases are slightly different from that of the wild protease as a result of different amino acid sequences, certain functional groups could be better accommodated by the active subsites of the mutant proteases than that of the wild protease.

The high activity sustenance of compound **21** (Figure 4) against all three HIV-1 mutants with different protease sequences remains to be a matter to discuss. To speculate the binding mode of compound **21** with HIV-1 protease, we constructed a molecular model using an X-ray crystal structure of compound **1** complex (PDB, 1hpx). On the basis of the design rationale of the D-amino acid containing Apns derivatives, the HMC of **21** ideally interacted with the two catalytic aspartic acids of wild-type HIV protease similar to that observed in **1**,[19,20] and the side chain of D-amino acid derivative was directed to the S_3 pocket while the *N*-acetylamino moiety was directed to the S_2 pocket. The common pose of **21** during the molecular dynamics (MD) simulations surrounded by explicit water was similar to that of **1** (Figure 5a). In the S_2 pocket, the carbonyl of the *N*-acetyl group could form hydrogen bond interactions with the two amide NH groups of Asp29 and Asp30 (Figure 5b). Additionally, the NH of the *N*-acetyl moiety could interact with the carbonyl of Gly48 from the flap region. This hydrogen bond network seemed to be a common feature among the D-amino acid containing analogues. Contrary to the hydrogen bond network, compound **1**'s hydrophobic side chain of methylthioalanine was tightly packed in the pocket space. On the other hand, the side chain of the D-amino acid residue of compound **21**, that is *p*-methoxybenzylthiomethyl, fitted in the S_3 pocket. The thioether of the side chain was flexibly bent to point to the S_3 pocket, letting the *p*-methoxybenzyl moiety contact favorably with the side chains of Arg8′, Pro81′, and Val82′ (Figure 5b).

The I84V mutation enlarges the space of the binding pockets with a volume of two methyl groups from I84V and I84′V than that of the wild-type enzyme. The acetylamino moiety of compound **21**, however, did not contact with the side chain of Ile84 in the S_2 pocket. Figure 5c shows one of the poses of **21** bound to the Val84 protease during an MD simulation. The rmsd from **21** in wild-type protease was 0.556 Å. The acetylamino moiety showed a slight contact with Val84 similar to the wild-type protease, keeping the aforementioned hydrogen bond network with amide NHs and CO of the protease backbone. Furthermore, slight rotations at the P_3 position kept the hydrophobic interactions seen in the wild-type protease. Of course, there are hydrophobic contacts within the S_2' pocket in which the rotation of the methylene moiety in P_2' 2-methyl-

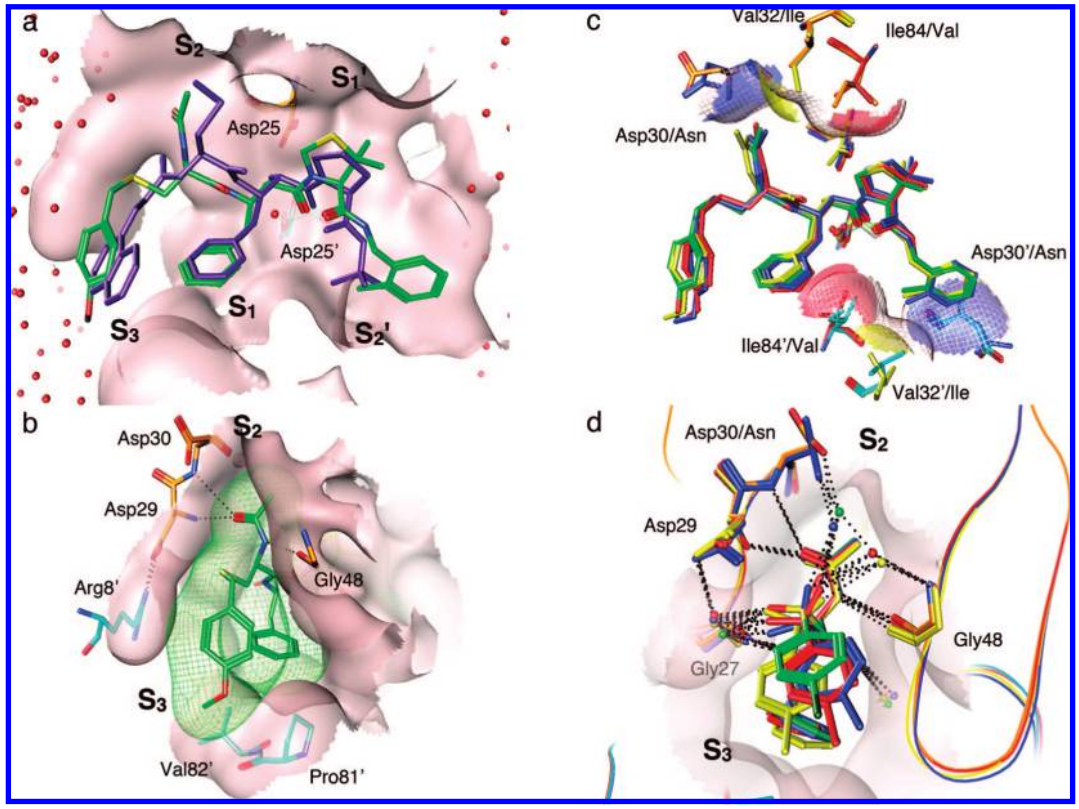


Figure 5. MD simulated poses of compound **21** (green stick) bound to HIV-1 proteases with a water soak. (a) Superimposition of compound **1** (purple stick, PDB 1hpx) in the wild-type protease. Flap regions of the protease are hidden. (b) Close-up view of the substituted D-cysteine residue of **21** with a green meshed molecular surface. (c) Overlay of four poses of **21** in the four proteases (wild-type, I84V (red), D30N (blue), V32I (yellow)). Mutated residues in each protease are shown with contact surfaces to **21** (meshed surface for wild-type). (d) Overlay of four poses of **25** in the four proteases with a close up view of S_2 and S_3 sites.

benzyl compensated for the change in I84V by a similar manner to an X-ray observation of compound **2** in V32F/I84V protease.[31] These observations suggest the reason why compound **21** could keep its activity against the RTV-resistant HIV-1 clone while compound **1** could not.

A mutation of Asp30 to Asn in the protease sequence represents the NFV-resistant strain. In this case, compound **21** became more sensitive to Asn than Asp. Molecular modeling experiments revealed a movement of the neutral amide side chain of Asn to contact with the hydrophobic acetylamino moiety of D-amino acid residue in the S_2 pocket, although the anionic carboxylate of Asp is directed toward the solvent, avoiding contact with the nonpolar acetyl group in the wild-type protease (Figure 5c). This additional hydrophobic contact between Asn and the acetyl moiety suggested an increased affinity in compound **21** while keeping the specific interactions of the D-amino acid residue described above (rmsd, 0.584 Å). The acetyl and mesyl moiety of other D-amino acid containing compounds can similarly contact with the side chain of Asn30, exhibiting an increased potency compared to that of the wild-type enzyme. In the S_2' pocket, the phenyl of the P_2' 2-methylbenzyl group interacted with the methylene side chain of the Asp or Asn residue.

In the case of the V32I mutation, as seen in the RTV/NFV-resistant clone, the space of the protease binding sites seemed to be relatively smaller. One of the poses of compound **21** slightly contacted with Val or Ile of the S_2 pocket during a simulation (Figure 5c). As observed in the I84V mutation, the hydrogen bond network between the D-amino acid residue and protease backbone of the S_2 pocket was kept, adopting the side chain in the S_3 pocket (rmsd, 0.398 Å). The side chain of Ile32′ in the S_2' pocket was inverted to accept the P_2' moiety from the inhibitor. Finally, the conformations of compound **21** in the wild-type protease were kept, even in the V32I protease.

Compound **25** was the only one to exhibit improvements in activity against all tested mutant HIVs. We constructed various molecular models of compound **25** bound to these mutated proteases (Figure 5d). The poses were similar to that of compound **21** except for the side chain of the D-amino acid residue. We observed two additional bridging water molecules between the inhibitor and the protease. One interacted between one of the sulfonyl oxygens and Gly27 NH or Asp29 carboxylate, while the other one was observed between another SO and Gly48 CO or Asp/Asn30. These stable interactions with water and the sulfonyl derivative could form a basis for the improvements in activity of compound **25**.

We compared the potency of D-amino acid containing inhibitors in the absence or presence of AGP at the same concentration as normal human serum (Table 3). The addition of AGP worsened the EC_{50} values of RTV and NFV to 18- and 29-fold, respectively. The potency of compound **1** also decreased 24-fold. The potency shifts of the D-amino acid containing analogues varied from 4- to 87-fold. A large shift in potency was observed in the case of compound **28**, which exhibited the highest activity among the series in the absence of AGP. On the other hand, compounds **12**, **17**, and **21** were relatively potent with moderate attenuation by the AGP addition (4.9-, 7.0-, 6.8-fold, respectively). These potency shifts are lower than that of the 12.7-fold of Lopinavir.[29] Lopinavir in combination with RTV is recommended for protease inhibitor-based regimens in antiretroviral naïve patients. As seen in compounds **12**, **17**, and **21**, a low AGP binding may increase the plasma concentration

Table 3. Anti-HIV-1 Activity in the Presence of AGP

compound	EC_{50}, nM		potency shift
	10% FCS[a]	10% FCS + AGP[b]	
9	25	155	6.2
10	20	90	4.5
11	20	278	14
12	18	88	4.9
13	16	96	6.0
16	14	110	7.9
17	12	84	7.0
20	9.4	94	10
21	13	89	6.8
25	27	1268	47
28	4.0	347	87
29	14	401	29
32	8.0	88	11
33	14	95	6.8
1	22	529	24
Ritonavir	24	422	18
Nelfinavir	21	606	29

[a] FCS, fetal calf serum. [b] AGP, α_1-acid glycoprotein.

of a free drug, reducing its dose and its undesired side effects such as lipodystrophy.

Conclusion

We synthesized a series of D-amino acid containing HIV protease inhibitors in order to apply the "D-amino acid concept" into an allophenynorstatine-based structure. Evaluation of these analogues suggested the usefulness of D-amino acid residues for high potency against not only the wild-type but also resistant HIV, especially the D30N mutation, in the protease. Several derivatives had promising results against all resistant HIVs and prospective profiles of cytotoxicity and AGP binding. These results suggest the potentials of the D-amino acid derived structure in the development of the next generation of HIV protease inhibitors.

Experimental Section

Commercial reagents and solvents were used without further purification. Column chromatography was performed on Merck Silica Gel 60 (70–230 mesh). Melting points were measured on a Yanagimoto micromelting apparatus without corrections. Analytical ^{1}H NMR spectra were recorded on a JEOL JNM-AL 300 FT NMR system with TMS as an internal standard. ES mass spectra were obtained from a Finnigan SSQ 7000 spectrometer. High-resolution FAB and EI mass spectra data were obtained on a JEOL JMS-SX 102A QQ and a JEOL JMS-GC MATE, respectively. Preparative HPLC was carried out on a C18 reverse phase column (20 mm × 250 mm; YMC Pack ODS SH343-5) with a binary solvent system: a linear gradient of CH_3CN in 0.1% aqueous TFA with a flow rate of 5.0 mL/min and detection at 230 nm. Analytical HPLC was performed using a C18 reverse-phase column (20 mm × 250 mm; YMC Pack ODS SH343–5) with binary solvent systems: (A) linier gradient of CH_3CN 40–100% in 0.1% aqueous TFA in 15 min, (B) liniar gradient of CH_3CN 20–80% in 0.1% aqueous TFA in 30 min at a flow rate of 0.9 mL/min, detected at 230 nm.

(*S*)-2-(*tert*-Butoxycarbonyl)amino-3-(4-methylbenzylthio)propanoic acid (5b) and (*S*)-2-(*tert*-Butoxycarbonyl)amino-3-(4-methylbenzylsulfonyl)propanoic acid (6b). To a solution of (*p*-methylphenyl)methanethiol (0.72 g, 5.80 mmol) in THF (30 mL) was added NaH (0.2 g, 0.49 mmol, 60% oil dispersion), and the mixture was stirred at room temperature for 10 min. A solution of *N*-*tert*-Boc-D-serine β-lactone (**4**) (1.14 g, 5.78 mmol) in THF (20 mL) was added dropwise over 10 min, and the mixture was additionally stirred at room temperature for 10 min. The reaction mixture was concentrated in vacuo and then purified by silica gel column chromatography ($CHCl_3$-MeOH) to give 1.59 g of compound **5b**; yield 88%. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.18 (d, J = 8.1 Hz, 2H), 7.09 (d, J = 7.9 Hz 2H), 6.44 (d, J = 7.0 Hz, 1H), 3.99–3.86 (m, 1H), 3.73–3.64 (m, 2H), 2.82 (dd, J = 13.4, 4.2 Hz, 1H), 2.66 (dd, J = 13.4 Hz, 7.3 Hz, 1H), 2.26 (s, 3H), 1.39 (s, 3H). MS (ES-) *m*/*z*: 340 for $[M - H]^-$.

The compound **5b** (500 mg, 1.54 mmol) was dissolved in $CHCl_3$ (20 mL), and then *m*-chloroperoxybenzoic acid (590 mg, 3.42 mmol) was added at 0 °C. The mixture was stirred at room temperature for 10 min. After the reaction mixture was concentrated in vacuo, purification of the residue by silica gel column chromatography ($CHCl_3$-MeOH) gave 480 mg of compound **6b**; yield 76% from thiol. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.75–7.63 (m, 1H), 7.27 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 8.1 Hz, 2H), 4.43 (s, 2H), 4.41–4.25 (m, 1H), 4.14 (dd, J = 5.6 Hz, 1.9 Hz, 1H), 3.49 (dd, J = 14.6 Hz, 3.4 Hz, 1H), 2.31 (s, 3H), 1.38 (s, 9H); MS (ES-) *m*/*z*: 396 for $[M - H]^-$.

(*S*)-2-(*tert*-Butoxycarbonyl)amino-3-(benzylsulfonyl)propanoic acid (6a). Compound **6a** was prepared from benzylmercaptan in a manner similar to that described for compound **6b**; yield 23%; mp 176–179 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.49–7.27 (m, 5H), 7.15 (br, 1H), 4.50 (s, 2H), 4.36 (br, 1H), 3.54 (d, J = 3.1 Hz, 1H), 3.50 (d, J = 2.8 Hz, 1H), 1.38 (s, 9H); MS (ES-) *m*/*z*: 342 for $[M - H]^-$.

(*S*)-2-(*tert*-Butoxycarbonyl)amino-3-(4-methoxybenzylsulfonyl)propanoic acid (6c). Compound **6c** was prepared from (*S*)-2-(*tert*-butoxycarbonylamino)-3-(4-methoxybenzylthio)propanoic acid in a manner similar to that described for compound **6b**; yield 94%. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.76–7.63 (m, 1H), 7.30 (d, 2H, J = 8.8 Hz), 6.95 (d, 2H, J = 8.6 Hz), 4.47–4.28 (m, 3H), 4.13 (dd, 1H, J = 5.6 Hz, 1.9 Hz), 3.76 (s, 1H), 3.48 (dd, 1H, J = 14.4 Hz, 3.2 Hz), 1.38 (s, 9H); MS (ES-) *m*/*z*: 356 for $[M - H]^-$.

(*S*)-2-(*tert*-Butoxycarbonyl)amino-3-(4-clorophenylsulfonyl)-propanoic acid (6e). Compound **6e** was prepared from 4-chlorobenzenethiol in a manner similar to that described for compound **6b**; yield 33%; mp 128–136 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.85 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 8.3 Hz, 2H), 6.94 (d, J = 7.2 Hz, 1H), 4.28–4.18 (m, 1H), 3.75–3.70 (m, 2H), 1.29 (s, 9H); MS (ES-) *m*/*z*: 362 for $[M - H]^-$.

(*S*)-2-(*tert*-Butoxycarbonyl)amino-3-(naphthalene-1-yl-sulfonyl)propanoic acid (6f). Compound **6f** was prepared from 1-thionaphthol in a manner similar to that described for compound **6b**; yield 82%; mp 133–142 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.58 (d, J = 8.3 Hz, 1H), 8.33 (d, J = 8.2 Hz, 1H), 8.16 (t, J = 8.0 Hz, 2H), 7.84–7.58 (m, 3H), 6.88 (br, 1H), 4.27 (br, 2H), 3.95–3.78 (m, 2H), 1.23 (s, 9H). MS (ES-) *m*/*z*: 378 for $[M - H]^-$.

(*S*)-2-(*tert*-Butoxycarbonyl)amino-3-(naphthalene-2-yl-sulfonyl)propanoic acid (6g). Compound **6g** was prepared from 2-naphthalenethiol in a manner similar to that described for compound **6b**; yield 30%; mp 107–113 °C. ^{1}H NMR (DMSO-d_6) δ(ppm): 8.52 (s, 1H), 8.16 (t, J = 8.1 Hz, 2H), 8.08 (d, J = 7.9 Hz, 1H), 7.85 (d, J = 9.3 Hz, 1H), 7.81–7.59 (m, 2H), 6.83 (d, J = 7.9 Hz, 1H), 4.33–4.22 (m, 1H), 3.85–3.67 (m, 2H), 1.11 (s, 9H). MS (ES-) *m*/*z*: 378 for $[M - H]^-$.

(*S*)-2-(*tert*-Butoxycarbonyl)amino-3-(quinoline-2-yl-sulfonyl)-propanoic acid (6h). Compound **6h** was prepared from 2-quinolinethiol in a manner similar to that described for compound **6b**; yield 57%; mp 115–123 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.76 (d, J = 8.6 Hz, 1H), 8.19 (d, J = 9.4 Hz, 2H), 8.07 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.91–7.79 (m, 1H), 6.96 (d, J = 7.3 Hz, 1H), 4.42–4.31 (m, 1H), 4.10–3.93 (m, 2H), 1.11 (s, 9H). MS (ES-) *m*/*z*: 379 for $[M - H]^-$.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(quinoline-2-yl-thio)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (36): General Method for *N*-Mesyl Derivatives. *N*-Boc-*S*-quinolinyl-D-cysteine **6h** (400 mg, 1.15 mmol) was dissolved in DMF (4 mL). The solution was cooled to 0 °C, and hydrocloride of allophenylnorstatine unit **7** (549 mg, 1.15 mmol), HOBt·H_2O (193 mg, 1.26 mmol), EDC·HCl(242 mg, 1.26 mmol), and triethylamine (240 μL, 1.72 mmol) were added. The mixture was stirred at room temperature for overnight, concentrated in vacuo, and diluted with EtOAc. The mixture was washed with 10% citric acid, 5% sodium

bicarbonate and brine, and then dried (Na_2SO_4) and concentrated in vacuo. The silica gel column chromatography gave 594 mg, yield 67%, of the Boc-protected intermediate. To the protected intermediate (400 mg, 0.52 mmol) were added anisole (85 μL, 0.78 mmol) and 4 N HCl/dioxane (2.6 mL), and the mixture was stirred at room temperature for 1 h. After the reaction mixture was concentrated in vacuo, ether was added. The precipitate was filtered and dried to give the hydrochloride salt (368 mg, quantitative). The amine (100 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) was treated with *N*-methylmorpholine (78.1 μL, 0.71 mmol) and methanesulfonyl chloride (43.8 μL, 0.57 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. After removal of the solvent in vacuo, the residue was dissolved in EtOAc, washed sequentially with 10% citric acid, 5% sodium bicarbonate, and brine, dried over Na_2SO_4, and concentrated in vacuo to give 62 mg, yield 59%. Purification of the product by preparative TLC ($CHCl_3$-MeOH) and preparative HPLC gave the title compound; yield 20% from **8**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.53 (d, J = 8.1 Hz, 1H), 8.42 (d, J = 5.7 Hz, 1H), 8.18 (d, J = 8.6 Hz, 1H), 7.93 (d, J = 8.6 Hz, 2H), 7.76 (d, J = 8.2 Hz, 1H), 7.52 (d, J = 7.4 Hz, 1H), 7.38–7.02 (m, 10H), 5.31 (d, J = 7.7 Hz, 1H), 5.08 (d, J = 9.2 Hz, 1H), 4.97 (d, J = 9.2 Hz, 1H), 4.52 (s, 1H), 4.50 (dd, J = 7.1 Hz, 2.4 Hz, 1H), 4.41 (dd, J = 15.4 Hz, 6.4 Hz, 1H), 4.32–4.16 (m, 3H), 3.22–3.15 (m, 2H), 2.87 (s, 3H), 2.80–2.57 (m, 2H), 2.27 (s, 3H), 1.51 (s, 3H), 1.37 (s, 3H). HRMS (FAB+) calcd for $C_{37}H_{44}N_5O_6S_3$ $[M + H]^+$, 750.2454; found, *m*/*z* 750.2448.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(quinoline-2-yl-thio)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (37): General Method for *N*-Acetyl Derivatives. To a solution of the amine intermediate mentioned above, hydrochloride salt of H-D-Cys(naphthalene-2-yl)-Apns-Dmt-NH-Bzl(2-Me) (100 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) and *N*-methylmorpholine (31 μL, 0.28 mmol) was added, followed by acetic anhydride (41 μL, 0.42 mmol), slowly under stirring at 0 °C. The resulting solution was stirred at room temperature for 1 h. After removal of the solvent in vacuo, the residue was dissolved in EtOAc, washed sequentially with 10% citric acid, 5% sodium bicarbonate, and brine, dried over Na_2SO_4, and concentrated in vacuo to give 54 mg, yield 54%. The crude product was purified by preparative TLC ($CHCl_3$-MeOH) and HPLC to give compound **37**; yield 44%. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.42 (t, J = 5.7 Hz, 1H), 8.33 (d, J = 9.0 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 8.6 Hz, 1H), 7.90 (d, J = 8.8 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.35–7.06 (m, 10H), 5.32 (d, J = 7.3 Hz, 1H), 5.07 (d, J = 9.0 Hz, 1H), 4.95 (d, J = 9.2 Hz, 1H), 4.70–4.62 (m, 6H), 4.52 (s, 1H), 4.50–4.35 (m, 2H), 4.22 (d, J = 5.0 Hz, 1H), 4.17 (d, J = 4.4 Hz, 1H), 3.47 (dd, J = 13.1 Hz, 4.7 Hz, 1H), 3.05 (dd, J = 13.4 Hz, 9.3 Hz, 1H), 2.82–2.63 (m, 2H), 2.27 (s, 3H), 1.79 (s, 3H), 1.51 (s, 3H), 1.36 (s, 3H). HRMS (FAB+) calcd for $C_{38}H_{44}N_5O_5S_2$ $[M + H]^+$, 714.2784; found, *m*/*z* 714.2789.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*R*)-2-methylsulfonylamino-4-phenylbutanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (8). Compound **8** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.51 (d, J = 8.4 Hz, 1H), 8.42 (t, J = 6.0 Hz, 1H), 7.37–6.98 (m, 14H), 5.14 (d, J = 9.4 Hz, 1H), 4.97 (d, J = 9.5 Hz, 1H), 4.53 (s, 1H), 4.48–4.39 (m, 2H), 4.27–4.16 (m, 2H), 3.91–3.81 (m, 1H), 2.78 (s, 3H), 2.73–2.65 (m, 2H), 2.27 (s, 3H), 2.21–2.12 (m, 2H), 1.60–1.47 (m, 5H), 1.37 (s, 3H). HRMS (FAB+) calcd for $C_{35}H_{45}N_4O_6S_2$ $[M + H]^+$, 681.2781; found, *m*/*z* 681.2793.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*R*)-2-acetylamino-4-phenylbutanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (9). Compound **9** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.39 (t, J = 5.4 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.33–6.95 (m, 14H), 5.13 (d, J = 9.2 Hz, 1H), 4.95 (d, J = 9.4 Hz, 1H), 4.52 (s, 1H), 4.47–4.34 (m, 3H), 4.22–4.16 (m, 2H), 2.73–2.63 (m, 2H), 2.27 (s, 3H), 2.23–2.12 (m, 2H), 1.84 (s, 3H), 1.63–1.43 (m, 5H), 1.36 (s, 3H). HRMS (FAB+) calcd for $C_{36}H_{45}N_4O_5S$ $[M + H]^+$, 645.3111; found, *m*/*z* 645.3120.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*R*)-3-benzyloxy-2-(methylsulfonylamino)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10). Compound **10** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.48 (d, J = 8.6 Hz, 1H), 8.41 (t, J = 5.6 Hz, 1H), 7.37–7.08 (m, 15H), 5.06 (d, J = 8.8 Hz, 1H), 4.98 (d, J = 9.2 Hz, 1H), 4.52 (s, 1H), 4.48–4.11 (m, 7H), 3.24 (d, J = 5.9 Hz, 2H), 2.82 (s, 3H), 2.75–2.63 (m, 2H), 2.27 (s, 3H), 1.51 (s, 3H), 1.36 (s, 3H). HRMS (EI+) calcd for $C_{35}H_{44}N_4O_7S_2$ $[M]^+$, 696.2651; found, *m*/*z* 696.2635.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*R*)-2-acetylamino-3-(benzyloxy)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (11). Compound **11** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.41 (t, J = 5.4 Hz, 1H), 8.36 (d, J = 8.7 Hz, 1H), 7.97 (d, J = 8.6 Hz, 1H), 7.35–7.08 (m, 14H), 5.08 (d, J = 9.5 Hz, 1H), 4.94 (d, J = 9.5 Hz, 1H), 4.66–4.57 (m, 1H), 4.51 (s, 1H), 4.47–4.38 (m, 2H), 4.27–4.09 (m, 4H), 3.27–3.18 (m, 2H), 2.73–2.62 (m, 2H), 2.27 (s, 3H), 1.84 (s, 3H), 1.50 (s, 3H), 1.35 (s, 3H). HR-MS (FAB^+) calcd for $C_{36}H_{45}N_4O_6S$ $[M + H]^+$, 661.3060; found, *m*/*z* 661.3069.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-3-benzylthio-2-(methylsulfonylamino)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12). Compound **12** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.48−8.37 (m, 2H), 7.47 (d, J = 9.7 Hz, 1H), 7.37–7.02 (m, 14H), 5.29 (d, J = 7.4 Hz, 1H), 5.07 (d, J = 9.0 Hz, 1H), 4.91 (d, J = 9.0 Hz, 1H), 4.52 (s, 1H), 4.49–4.34 (m, 2H), 4.22 (d, J = 4.8 Hz, 1H), 4.17 (d, J = 4.6 Hz, 1H), 3.94 (d, J = 5.7 Hz, 1H), 3.77 (s, 2H), 3.17 (d, J = 5.1 Hz, 1H), 2.80–2.56 (m, 3H), 2.39 (s, 3H), 2.26 (s, 3H), 1.49 (s, 3H), 1.36 (s, 3H). HRMS (FAB+) calcd for $C_{35}H_{44}N_4O_6S_3Na$ $[M + Na]^+$, 735.2321; found, *m*/*z* 735.2327.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(benzylthio)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13). Compound **13** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.36 (d, J = 5.5 Hz, 1H), 8.05 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.35–7.06 (m, 14H), 5.33 (d, J = 7.1 Hz, 1H), 5.01 (d, J = 9.0 Hz, 1H), 4.91 (d, J = 9.2 Hz, 1H), 4.49 (s, 1H), 4.47–4.34 (m, 3H), 4.20 (d, J = 4.8 Hz, 1H), 4.15 (d, J = 4.4 Hz, 1H), 3.71 (s, 1H), 3.17 (d, J = 5.1 Hz, 1H), 2.80–2.60 (m, 3H), 2.26 (s, 3H), 1.82 (s, 3H), 1.49 (s, 3H), 1.34 (s, 3H). HRMS (FAB+) calcd for $C_{36}H_{45}N_4O_5S_2$ $[M + H]^+$, 677.2831; found, *m*/*z* 677.2827.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-3-benzylsulfonyl-2-(methylsulfonylamino)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (14). Compound **14** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.59 (d, J = 8.4 Hz, 1H), 8.42 (t, J = 4.8 Hz, 1H), 7.74 (br, 1H), 7.47–7.03 (m, 14H), 5.42 (d, J = 7.0 Hz, 1H), 5.01 (d, J = 9.2 Hz, 1H), 4.97 (d, J = 9.3 Hz, 1H), 4.52 (s, 1H), 4.48 (dd, J = 7.4 Hz, 3.0 Hz, 1H), 4.45–4.34 (m, 4H), 4.23 (d, J = 4.6 Hz, 1H), 4.18 (d, J = 5.0 Hz, 1H), 3.16–3.23 (m, 1H), 3.02–2.97 (m, 1H), 2.88 (s, 3H), 2.80–2.60 (m, 2H), 2.26 (s, 3H), 1.51 (s, 3H), 1.36 (s, 3H). HRMS (FAB+) calcd for $C_{35}H_{44}N_4O_8S_3Na$ $[M + Na]^+$, 767.2219; found, *m*/*z* 767.2214.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(benzylsulfonyl)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (15). Compound **15** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.44 (t, J = 5.4 Hz, 1H), 8.27 (d, J = 9.1 Hz, 1H), 8.24 (d, J = 8.8 Hz, 1H), 7.42–7.03 (m, 14H), 5.39 (d, J = 7.5 Hz, 1H), 5.01 (d, J = 8.8 Hz, 1H), 4.92 (d, J = 9.0 Hz, 1H), 4.89–4.78 (m, 1H), 4.50 (s, 1H), 4.47–4.38 (m, 2H), 4.34 (d, J = 8.4 Hz, 2H), 4.18 (d, J = 5.4 Hz, 1H), 4.13 (d, J = 4.4 Hz, 1H), 3.24–3.13 (m, 1H), 2.94 (dd, J = 14.7 Hz, 9.6 Hz, 1H), 2.86–2.62 (m, 2H), 2.25 (s, 3H), 1.87 (s,

3H), 1.49 (s, 3H), 1,35 (s, 3H). HRMS (FAB+) calcd for $C_{36}H_{44}N_4O_7S_2Na$ $[M + Na]^+$, 731.2549; found, *m/z* 731.2562.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(4-methylbenzylthio)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (16). Compound **16** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.66 (d, $J = 7.5$ Hz, 1H), 8.44(t, $J = 6.0$ Hz, 1H), 7.43 (d, $J = 9.2$ Hz, 1H), 7.33–7.09 (m, 13H), 5.09 (d, $J = 8.8$ Hz, 1H), 4.96 (d, $J = 9.2$ Hz, 1H), 4.53 (s, 1H), 4.49–4.39 (m, 2H), 4.23–4.02 (m, 3H), 3.62 (d, $J = 13.2$ Hz, 1H), 3.53 (d, $J = 13.2$ Hz, 1H), 2.84 (s, 3H), 2.73–2.65 (m, 2H), 2.32–2.18 (m, 8H, overlapped with DMSO), 1.51 (s, 3H), 1.32 (s, 3H). HRMS (FAB+) calcd for $C_{36}H_{46}N_4O_6S_3Na$ $[M + Na]^+$, 749.2477; found, *m/z* 749.2487.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(4-methylbenzylthio)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (17). Compound **17** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.58 (d, $J = 9.2$ Hz, 1H), 8.42 (br, 1H), 7.98 (d, $J = 9.7$ Hz, 1H), 7.30–7.09 (m, 13H), 5.09 (d, $J = 8.8$ Hz, 1H), 4.94 (d, $J = 9.2$ Hz, 1H), 4.68–4.54 (m, 1H), 4.52 (s, 1H), 4.46–4.38 (m, 2H), 4.24–4.09 (m, 2H), 3.55 (br, 2H), 2.73–2.27 (m, 2H), 2.37–2.10 (m, 8H, overlapped with DMSO), 1.81 (s, 3H), 1.50 (s, 3H), 1.32 (s, 3H). HRMS (FAB+) calcd for $C_{37}H_{47}N_4O_5S_2$ $[M + H]^+$, 691.2988; found, *m/z* 691.2979.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(4-methylbenzylsulfonyl)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (18). Compound **18** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.57 (d, $J = 8.6$ Hz, 1H), 8.41 (t, $J = 5.4$ Hz, 1H), 7.72 (d, $J = 9.4$ Hz, 1H), 7.32–7.10 (m, 13H), 5.01 (d, $J = 9.0$ Hz, 1H), 4.97 (d, $J = 9.0$ Hz, 1H), 4.52 (s, 1H), 4.47 (d, $J = 3.0$ Hz, 1H), 4.43–4.34 (m, 4H), 4.20 (dd, $J = 15.0$ Hz, 4.8 Hz, 2H), 3.06–2.91 (m, 2H), 2.87 (s, 3H), 2.82–2.61 (m, 2H), 2.31 (s, 3H), 2.26 (s, 3H), 1.51 (s, 3H), 1.36 (s, 3H). HRMS (FAB+) calcd for $C_{36}H_{46}N_4O_8S_3Na$ $[M + Na]^+$, 781.2375; found, *m/z* 781.2380.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(4-methylbenzylsulfonyl)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (19). Compound **19** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.43 (t, $J = 5.4$ Hz, 1H), 8.25 (d, $J = 9.0$ Hz, 1H), 8.21 (d, $J = 10.8$ Hz, 1H), 7.31–7.11 (m, 13H), 5.01 (d, $J = 8.8$ Hz, 1H), 4.92 (d, $J = 9.0$ Hz, 1H), 4.90–4.80 (m, 1H), 4.50 (s, 1H), 4.48–4.23 (m, 4H), 4.15 (dd, $J = 14.7$ Hz, 4.8 Hz, 2H), 3.17–3.12 (m, 1H), 2.99–2.91 (m, 1H), 2.85–2.58 (m, 2H), 2.28 (s, 3H), 2.25 (s, 3H), 1.86 (s, 3H), 1.49 (s, 3H), 1.34 (s, 3H). HRMS (FAB+) calcd for $C_{37}H_{47}N_4O_7S_2$ $[M + H]^+$, 723.2886; found, *m/z* 723.2878.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(4-methoxybenzylthio)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (20). Compound **20** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.65 (d, $J = 8.8$ Hz, 1H), 8.43 (t, 1H, $J = 5.1$ Hz, 1H), 7.44 (d, $J = 9.5$ Hz, 1H), 7.33–7.10 (m, 11H), 6.86 (d, $J = 8.6$ Hz, 2H), 5.10 (d, $J = 9.2$ Hz, 1H), 4.96 (d, $J = 9.4$ Hz, 1H), 4.53 (s, 1H), 4.49 (br, 1H), 4.42 (dd, $J = 15.2$ Hz, 5.9 Hz, 1H), 4.28–4.16 (m, 2H), 4.09–4.00 (m, 1H), 3.73 (s, 3H), 3.63–3.50 (m, 2H), 2.84 (s, 3H), 2.73–2.10 (m, 2H), 2.33–2.19 (m, 5H), 1.51 (s, 3H), 1.36 (s, 3H). HRMS (FAB+) calcd for $C_{36}H_{47}N_4O_7S_3$ $[M + H]^+$, 743.2607; found, *m/z* 743.2610.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(4-methoxybenzylthio)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (21). Compound **21** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.48 (d, $J = 8.1$ Hz, 1H), 8.42 (t, $J = 5.4$ Hz, 1H), 7.98 (d, $J = 8.7$ Hz, 1H), 7.33–7.09 (m, 11H), 6.86 (d, $J = 8.4$ Hz, 2H), 5.09 (d, $J = 9.0$ Hz, 1H), 4.94 (d, $J = 9.3$ Hz, 1H), 4.64–4.38 (m, 1H), 4.52 (s, 1H), 4.46–4.38 (m, 2H), 4.22–4.13 (m, 2H), 3.73 (s, 3H), 3.54 (d, $J = 2.7$ Hz, 2H), 2.72–2.62 (m, 2H), 2.35–2.27 (m, 4H), 2.14 (dd, $J = 13.5$ Hz, 9.3 Hz, 1H), 1.84 (s, 3H), 1.50 (s, 3H), 1.35 (s, 3H). HRMS (FAB+) calcd for $C_{37}H_{47}N_4O_6S_2$ $[M + H]^+$, 707.2937; found, *m/z* 707.2949.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(4-methoxybenzylsulfonyl)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (22). Compound **22** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 9.28 (d, $J = 9.3$ Hz, 1H), 8.63 (d, $J = 8.6$ Hz, 2H), 8.56 (d, $J = 8.8$ Hz, 1H), 8.40 (m, 1H), 7.32–7.12 (m, 13H), 5.02–4.98 (m, 2H), 4.51–4.17 (m, 7H), 3.76 (s, 3H), 3.00–2.26 (m, 4H), 2.88 (s, 3H), 2.27 (s, 3H), 1.51 (s, 3H), 1.36 (w, 3H). HR-MS (FAB^+) calcd for $C_{36}H_{47}N_4O_9S_3$ $[M + H]^+$, 775.2505; found, *m/z* 775.2499.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(4-methoxybenzylsulfonyl)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (23). Compound **23** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.44 (t, $J = 5.1$ Hz, 1H), 8.27–8.22 (m, 2H), 7.29–7.13 (m, 11H), 6.91 (d, $J = 8.4$ Hz, 2H), 5.01 (d, $J = 9.3$ Hz, 1H), 4.92 (d, $J = 8.7$ Hz, 1H), 4.86–4.78 (m, 1H), 4.50 (s, 1H), 4.42–4.11 (m, 4H), 3.74 (s, 3H), 3.15–3.06 (m, 1H), 2.93 (dd, $J = 14.0$ Hz, 9.2 Hz, 1H), 2.84–2.64 (m, 2H), 2.25 (s, 3H), 1.86 (s, 3H), 1.49 (s, 3H), 1.34 (s, 3H). HRMS (FAB+) calcd for $C_{37}H_{47}N_4O_8S_2$ $[M + H]^+$, 739.2835; found *m/z* 739.2823.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(4-chlorophenylsulfonyl)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (24). Compound **24** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.58 (d, $J = 8.3$ Hz, 1H), 8.40 (t, $J = 4.9$ Hz, 1H), 7.81 (d, $J = 8.8$ Hz, 2H), 7.70 (d, $J = 8.6$ Hz, 2H), 7.49–7.05 (m, 10H), 5.37 (d, $J = 7.5$ Hz, 1H), 4.98 (d, $J = 9.0$ Hz, 1H), 4.93 (d, $J = 9.2$ Hz, 1H), 4.50 (s, 1H), 4.48–4.34 (m, 2H), 4.26 (d, $J = 7.5$ Hz, 1H), 4.22 (d, $J = 5.1$ Hz, 1H), 4.17 (d, $J = 5.0$ Hz, 1H), 3.17–3.01 (m, 2H), 2.76 (s, 3H), 2.74–2.57 (m, 2H), 2.26 (s, 3H), 1.49 (s, 3H), 1.35 (s, 3H). HRMS (FAB+) calcd for $C_{34}H_{41}ClN_4O_8S_3Na$ $[M + Na]^+$, 787.1673; found, *m/z* 787.1678.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(4-chlorophelysulfonyl)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (25). Compound **25** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.44 (t, $J = 5.5$ Hz, 1H), 8.18 (d, $J = 9.2$ Hz, 1H), 7.97 (d, $J = 9.0$ Hz, 1H), 7.74 (d, $J = 9.0$ Hz, 2H), 7.70 (d, $J = 9.0$ Hz, 2H), 7.32–7.04 (m, 9H), 5.37 (d, $J = 7.4$ Hz, 1H), 4.97 (d, $J = 9.2$ Hz, 1H), 4.89 (d, $J = 9.0$ Hz, 1H), 4.69–4.54 (m, 1H), 4.48 (s, 1H), 4.43–4.33 (m, 2H), 4.19 (d, $J = 4.9$ Hz, 1H), 4.14 (d, $J = 4.6$ Hz, 1H), 3.22–3.16 (m, 2H), 2.80–2.57 (m, 2H), 2.25 (s, 3H), 1.64 (s, 3H), 1.49 (s, 3H), 1.34 (s, 3H). HR-MS (FAB^+) calcd for $C_{35}H_{41}ClN_4O_7S_2$ Na $[M + Na]^+$, 751.2003; found, *m/z* 751.2009.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(4-fluorophenylsulfonyl)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (26). Compound **26** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.58 (d, $J = 9.2$ Hz, 1H), 8.41 (t, $J = 5.5$ Hz, 1H), 7.89 (d, $J = 5.3$ Hz, 1H), 7.86 (d, $J = 5.1$ Hz, 1H), 7.47 (t, 2H, $J = 8.7$ Hz 2H), 7.33–7.18 (m, 3H), 7.17–7.01 (m, 7H), 5.37 (d, $J = 7.1$ Hz, 1H), 4.98 (d, $J = 9.5$ Hz 1H), 4.93 (d, $J = 9.0$ Hz, 1H), 4.50 (s, 1H), 4.48–4.32 (m, 2H), 4.27 (dd, $J = 9.6$ Hz, 3.2 Hz, 1H), 4.21 (d, $J = 4.6$ Hz 1H), 4.17 (d, $J = 4.4$ Hz, 1H), 3.17 (dd, $J = 9.9$, 4.8 Hz 1H), 2.99 (dd, $J = 14.8$ Hz, 3.8 Hz, 1H), 2.76 (s, 3H), 2.74–2.57 (m, 2H), 2.26 (s, 3H), 1.49 (s, 3H), 1.35 (s, 3H). HRMS (FAB+) calcd for $C_{34}H_{42}FN_4O_8S_3$ $[M + H]^+$, 749.2149; found, *m/z* 749.2140.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(4-fluoropheylsulfonyl)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (27). Compound **27** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.44 (t, J = 5.4 Hz, 1H), 8.18 (d, J = 8.8 Hz, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.82 (d, J = 5.3 Hz, 1H), 7.79 (d, J = 5.1 Hz, 1H), 7.47 (t, J = 8.8 Hz, 2H), 7.34–7.03 (m, 9H), 5.38 (d, J = 7.5 Hz, 1H), 4.97 (d, J = 9.2 Hz, 1H), 4.89 (d, J = 9.2 Hz, 1H), 4.70–4.53 (m, 1H), 4.48 (s, 1H), 4.44–4.31 (m, 2H), 4.20–4.09 (m, 2H), 3.22–3.15 (m, 2H), 2.80–2.58 (m, 2H), 2.25 (s, 3H), 1.65 (s, 3H), 1.49 (s, 3H), 1.34 (s, 3H). HRMS (FAB+) calcd for $C_{35}H_{42}FN_4O_7S_2$ [M + H]$^+$, 713.2479; found, *m*/*z* 713.2487.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(naphthalene-1-yl-thio)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (28). Compound **28** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.66 (d, J = 8.4 Hz, 1H), 8.45–8.38 (m, 1H), 8.16 (d, J = 9.3 Hz, 1H), 7.97 (d, J = 6.9 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.66–6.99 (m, 14H), 5.08 (d, J = 9.4 Hz, 1H), 4.97 (d, J = 9.0 Hz, 1H), 4.52 (s, 1H), 4.49 (d, J = 2.4 Hz, 1H), 4.44–4.08 (m, 4H), 2.84 (d, J = 7.5 Hz, 2H), 2.81 (s, 3H), 2.73–2.65 (m, 2H), 2.27 (s, 3H), 1.51 (s, 3H), 1.36 (s, 3H). HR-MS (FAB$^+$) calcd for $C_{38}H_{45}N_4O_6S_3$ [M + H]$^+$, 749.2501; found, *m*/*z* 749.2515.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(naphthalene-1-yl-thio)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (29). Compound **29** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.46–8.38 (m, 2H), 8.14 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 9.3 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.60–7.01 (m, 14H), 5.07 (d, J = 8.6 Hz, 1H), 4.94 (d, J = 9.7 Hz, 1H), 4.62–4.53 (m, 1H), 4.51 (s, 1H), 4.45–4.37 (m, 2H), 4.22–4.09 (m, 2H), 2.94–2.63 (m, 5H), 2.26 (s, 3H), 1.82 (s, 3H), 1.50 (s, 3H), 1.36 (s, 3H). HRMS (FAB+) calcd for $C_{39}H_{45}N_4O_5S_2$ [M + H]$^+$, 713.2831; found, *m*/*z* 713.2838.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(naphthalene-1-yl-sulfonyl)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (30). Compound **30** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.64–8.58 (m, 2H), 8.41- 8.36 (m, 2H), 8.20 (d, J = 8.1 Hz, 1H), 8.11 (d, J = 7.5 Hz, 1H), 7.84–7.68 (m, 3H), 7.58 (br, 1H), 7.30–7.27 (m, 1H), 7.19–6.96 (m, 5H), 6.77–6.57 (m, 3H), 5.33 (d, J = 7.3 Hz, 1H), 4.95 (d, J = 9.0 Hz, 1H), 4.91 (d, J = 9.5 Hz, 1H), 4.52 (dd, J = 9.7 Hz, 3.5 Hz, 1H), 4.49 (s, 1H), 4.46–4.30 (m, 2H), 4.20 (d, J = 5.0 Hz, 1H), 4.15 (d, J = 4.8 Hz, 1H), 3.17 (d, J = 5.3 Hz, 1H), 3.05 (dd, J = 14.1 Hz, 3.5 Hz, 1H), 2.81 (s, 3H), 2.24 (s, 3H), 1.48 (s, 3H), 1.34 (s, 3H). HRMS (FAB+) calcd for $C_{38}H_{45}N_4O_8S_3$ [M + H]$^+$, 781.2400; found, *m*/*z* 781.2396.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(naphthalene-1-yl-sulfonyl)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (31). Compound **31** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.53 (d, J = 8.1 Hz, 1H), 8.39 (t, J = 5.3 Hz, 1H), 8.34 (d, J = 8.4 Hz, 1H), 8.18 (t, J = 7.2 Hz, 2H), 8.05 (dd, J = 7.5 Hz, 1.1 Hz, 1H), 7.99–7.91 (m, 1H), 7.83–7.63 (m, 3H), 7.31–7.21 (m, 1H), 7.13–6.97 (m, 5H), 6.95–6.80 (m, 3H), 5.33 (d, J = 7.5 Hz, 1H), 4.93 (d, J = 9.2 Hz, 1H), 4.87 (d, J = 9.0 Hz, 1H), 4.80 (dd, J = 15.4 Hz, 7.0 Hz, 1H), 4.45 (s, 1H), 4.42–4.27 (m, 2H), 4.17–4.05 (m, 2H), 3.61–3.44 (m, 1H, overlapped with H_2O), 3.17 (d, J = 5.1 Hz, 2H), 2.77–2.57 (m, 2H), 2.22 (s, 3H), 1.56 (s, 3H), 1.47 (s, 3H), 1.31 (s, 3H). HRMS (FAB+) calcd for $C_{39}H_{45}N_4O_7S_2$ [M + H]$^+$, 745.2729; found, *m*/*z* 745.2723.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(naphthalene-2-yl-thio)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (32). Compound **32** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.72 (d, J = 8.6 Hz, 1H), 8.43 (t, J = 5.2 Hz, 1H), 7.98–7.80 (m, 3H), 7.72 (s, 1H), 7.63 (br, 1H), 7.59–7.44 (m, 2H), 7.40–7.27 (m, 4H), 7.25–7.00 (m, 6H), 5.29 (d, J = 7.4 Hz, 1H), 5.09 (d, J = 9.2 Hz, 1H), 4.97 (d, J = 9.2 Hz, 1H), 4.52 (s, 1H), 4.50 (dd, J = 7.2 Hz, 2.7 Hz, 1H), 4.41 (dd, J = 14.8 Hz, 6.4 Hz, 1H), 4.14–4.05 (m, 1H), 2.95–2.86 (m, 2H), 2.83 (s, 3H), 2.79–2.57 (m, 2H), 2.27 (s, 3H), 1.51 (s, 3H), 1.37 (s, 3H). HR-MS (FAB$^+$) calcd for $C_{38}H_{45}N_4O_6S_3$ [M + H]$^+$, 749.2501; found, *m*/*z* 749.2507.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(naphthalene-2-yl-thio)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (33). Compound **33** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.54 (d, J = 8.4 Hz, 1H), 8.41 (d, J = 5.5 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 7.95–7.83 (m, 16H), 7.73 (s, 1H), 7.58–7.42 (m, 2H), 7.39–7.25 (m, 4H), 7.24–7.02 (m, 6H), 5.26 (d, J = 7.3 Hz, 1H), 5.08 (d, J = 9.2 Hz, 1H), 4.94 (d, J = 8.8 Hz, 1H), 4.66–4.54 (m, 1H), 4.51 (s, 1H), 4.50–4.35 (m, 2H), 4.21 (d, J = 4.8 Hz, 1H), 4.16 (d, J = 4.4 Hz, 1H), 2.94 (dd, J = 13.2 Hz, 4.8 Hz, 1H), 2.82 (dd, J = 13.0 Hz, 9.3 Hz, 1H), 2.77–2.61 (m, H), 2.27 (s, 3H), 1.81 (s, 3H), 1.51 (s, 3H), 1.36 (s, 3H). HRMS (FAB+) calcd for $C_{39}H_{45}N_4O_5S_2$ [M + H]$^+$, 713.2831; found, *m*/*z* 713.2823.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(naphthalene-2-yl-sulfonyl)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (34). Compound **34** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.58–7.86 (m, 7H), 7.82–6.93 (m, 12H), 4.97 (d, J = 8.6 Hz, 1H), 4.91 (d, J = 8.8 Hz, 1H), 4.49 (s, 1H), 4.43–4.16 (m, 5H), 2.76 (s, 3H), 2.73–2.64 (m, 4H, overlapped with DMSO), 2.25 (s, 3H), 1.49 (s, 3H), 1.34 (s, 3H). HRMS (FAB+) calcd for $C_{38}H_{45}N_4O_8S_3$ [M + H]$^+$, 781.2400; found, *m*/*z* 781.2394.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(naphthalene-2-yl-sulfonyl)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (35). Compound **35** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.50–8.34 (m, 2H), 8.22–8.04 (m, 2H), 7.94 (d, J = 8.8 Hz, 1H), 7.81–7.65 (m, 3H), 7.32–6.95 (m, 9H), 5.36 (d, J = 7.3 Hz, 1H), 4.95 (d, J = 9.0 Hz, 1H), 4.87 (d, J = 9.5 Hz, 1H), 4.73–4.60 (m, 1H), 4.46 (s, 1H), 4.41–4.29 (m, 2H), 4.19–4.07 (m, 2H), 3.51–3.16 (m, 2H, overlapped with DMSO), 2.79–2.57 (m, 2H), 2.22 (s, 3H), 1.47 (s, 6H), 1.32 (s, 3H). HR-MS (FAB$^+$) calcd for $C_{39}H_{45}N_4O_7S_2$ [M + H]$^+$, 745.2730; found, *m*/*z* 745.2726.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-((2*S*)-3-(quinoline-2-yl-sulfonyl)-2-(methylsulfonylamino)propanoyl)amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (38). Compound **38** was prepared from compound **7** in a manner similar to that described for compound **36**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.72 (d, J = 8.6 Hz, 1H), 8.57 (d, J = 8.8 Hz, 1H), 8.40 (t, J = 5.6 Hz, 1H), 8.18 (d, J = 8.8 Hz, 2H), 7.97 (t, J = 7.6 Hz, 2H), 7.83 (t, J = 7.6 Hz, 1H), 7.38–6.95 (m, 10H), 5.36 (d, J = 7.5 Hz, 1H), 4.94 (d, J = 9.2 Hz, 1H), 4.89 (d, J = 9.2 Hz, 1H), 4.49 (s, 1H), 4.47–4.33 (m, 3H), 4.22 (d, J = 4.7 Hz, 1H), 4.17 (d, J = 4.8 Hz, 1H), 3.61–3.49 (m, 1H), 3.17 (d, J = 5.1 Hz, 1H), 2.81–2.57 (m, 5H), 2.25 (s, 3H), 1.49 (s, 3H), 1.34 (s, 3H). HRMS (FAB+) calcd for $C_{37}H_{43}N_5O_8S_3Na$ [M + Na]$^+$, 804.2171; found, *m*/*z* 804.2177.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-((2*S*)-2-acetylamino-3-(quinoline-2-yl-sulfonyl)propanoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (39). Compound **39** was prepared from compound **7** in a manner similar to that described for compound **37**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.73 (d, J = 8.4 Hz, 1H), 8.44 (t, J = 5.3 Hz, 1H), 8.22–8.07 (m, 3H), 8.00 (d, J = 9.3 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.83 (t, J = 7.5 Hz, 1H), 7.34–6.99 (m, 10H), 5.36 (d, J = 7.7 Hz, 1H), 4.94 (d, J = 8.8 Hz, 1H), 4.86 (d, J = 9.4 Hz, 1H), 4.82–4.70 (m, 1H), 4.47 (s, 1H), 4.32–4.44 (m, 2H), 4.20 (d, J = 4.6 Hz, 1H), 4.15 (d, J = 5.0 Hz, 1H), 3.56 (d, J = 6.0 Hz, 1H), 3.17 (d, J = 5.1 Hz, 1H), 2.82–2.56 (m, 2H), 2.22 (s, 3H), 1.48 (s, 6H), 1.33 (s, 3H). HRMS (FAB+) calcd for $C_{38}H_{44}N_5O_7S_2$ [M + H]$^+$, 746.2682; found, *m*/*z* 746.2687.

HIV-1 Protease Inhibition. In the HIV protease assay, 25 μL of 200 mM 2-(*N*-morpholino)ethanesulfonic acid (MES)-NaOH buffer (pH 5.5), containing 2 mM dithiothreinol (DDT), 2 mM EDTA-2Na, and 1 M NaCl, was mixed with 5 μL of inhibitor (10 nM) dissolved in DMSO and 10 μL of HIV-1 protease (2 μg/mL) in 50 mM AcOH (pH 5.5), containing 1 mM EDTA-2Na, 25 mM NaCl, 0.2% mercaptoethanol, 0.2% Nonidet P-40, and 15% glycerol. The mixture of was preincubated for 5 min at 37 °C, and the enzymatic reaction was initiated by addition of 10 μL of a 75 mM substrate solution (H-Lys-Ala-Arg-Val-Tyr*Phe(*p*-NO_2)-Glu-Ala-Nle-NH_2, synthesized by a conventional solid-phase method). After incubation for 60 min at 37 °C, the reaction was terminated by addition of 75 μL of 1N HCl. The C-terminal cleavage fragment (H-Phe(*p*-NO_2)-Glu-Ala-Nle-NH_2) was separated by reverse-phase HPLC on a C18 column with linear gradient of 0.1% aqueous TFA to CH_3CN, detected by monitoring the fluorescence intensity. Percent inhibition was obtained compared to intensity without inhibitor.

Antiviral Assays. The anti-HIV activity was assayed by determining the level of inhibition of virus-induced cytopathic effect in the cells as previously described.[32] HIV-1 IIIB, kindly provided by Prof. Harada, was used for anti-HIV assay. MT-4 cells were added to each well of a 96-well flat-bottom plate containing serial 2-fold dilution of test compounds in duplicates. HIV-1 was added to each well. Plates were incubated for 4 day in a CO_2 incubator at 37 °C. After treatment with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), the optical density of plates were measured (560 nm) and percent cytopathic effect reduction was calculated, then EC_{50} values were estimated by fitting the data to a median-effect equation. Cytotoxicity was determined by incubation in the absence of the virus. Antiviral assay in the presence of α_1-acid glycoprotein (AGP) was performed using similar method with or without 1 mg/mL of AGP.

Protease resistant viruses were isolated from HIV-1 IIIB. Briefly, HIV-1 IIIB was infected with M8166 cells kindly provided by Prof. Hayami and the infected cells were cultured in the presence of protease inhibitors. Protease coding regions of the resistant viruses were amplified by PCR and then subcloned to pNL-432 clone, kindly provided by Prof. Adachi. Complete protease sequences of the protease genes were confirmed by DNA sequencing. The viral supernatants were propagated, titered, and utilized for MTT assay in M8166 cells in a same manner mentioned above.

Molecular Dynamics Simulation of HIV Protease Binding Models. Initial conformation of inhibitor was constructed based on an X-ray crystal structure of compound **1** with wild-type HIV-1 protease (PDB, 1HPX) using the Molecular Operating Environment modeling package (MOE 2006.08, Chemical Computing Group, Inc., Montreal, Canada) with a MMFF94x force field. A carboxyl group of an Asp25 close to the HMC carbonyl group was protonated. The binding area was immersed in a 20 Å sphere of TIP3P water, centered on an oxygen atom of structural water between inhibitor and the enzyme flap. The inhibitor, contacted residues and flap region of the enzyme, and surrounded water were energy-minimized while the other atoms were fixed. MD simulations were performed without heating at 298 K for 300 ps equilibration with 1.5 fs time step. An additional 50 ps of MD simulation was performed for data collection. Two residues in the protease dimer were mutated from 1HPX to model each mutant protease of the three drug-resistant HIV-1 clones, and energy-minimizations and MD simulations were performed as described above.

Acknowledgment. This research was supported in parts by the Frontier Research Program and the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, the Japan Health Sciences Foundation, and Japan Society for Promotion of Science's Post-Doctoral Fellowship for Foreign Researchers. We gratefully acknowledge the assistance of T. Ito and H. Tsukamoto for determining enzyme inhibition.

Supporting Information Available: Results of HPLC analysis of final compounds. This is available free of charge via the Internet at http://pubs.acs.org.

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JM701555P