Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 1299–1308
Bioorganic & Medicinal Chemistry

Structure–activity relationships of novel HIV-1 protease inhibitors containing the 3-amino-2-chlorobenzoyl-allophenylnorstatine structure

Tsutomu Mimoto,* Satoshi Nojima, Keisuke Terashima, Haruo Takaku, Makoto Shintani and Hideya Hayashi

Drug Research Division, Dainippon Sumitomo Pharma Co., Ltd., Enoki 33-94, Suita, Osaka 564-0053, Japan

Received 26 September 2007; revised 17 October 2007; accepted 18 October 2007
Available online 23 October 2007

Abstract—A series of peptidomimetic human immunodeficiency virus (HIV) protease inhibitors containing substituted allophenylnorstatine (Apns: (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid) were designed and synthesized. From the structure–activity relationship of this series of compounds, SM-309515 was found to have potent antiviral activity against wild-type and resistant HIV-1s and to possess a desirable pharmacokinetic profile in dogs.

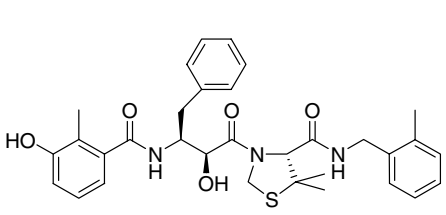
1. Introduction

Human immunodeficiency virus (HIV) protease is essential for viral replication and is the main target for approved antiviral drugs. Combination chemotherapy with reverse transcriptase inhibitors and protease inhibitors (PI) has proven to be highly effective in suppressing viral replication to undetectable levels.[1] Despite such remarkable achievement, the use of current antiretroviral regimens continues to be limited by complexity, tolerability, drug resistance, and cross-resistance. In addition, non-adherence to such regimens can lead to reduced effectiveness and increased drug resistance. Although newer drugs offer improvements over existing agents by having simpler dosing schedules, better tolerability, and/or improved antiviral activity, one of the most desirable properties of any novel protease inhibitor is that it can be dosed once daily. Atazanavir,[2] the first once-daily protease inhibitor, has strong anti-HIV efficacy and favorable pharmacokinetic profile. However, when used for salvage therapy in patients with a degree of resistance, atazanavir is combined with ritonavir, which is known to sustain high plasma drug levels by inhibiting cytochrome P450 (CYP) 3A4-mediated drug metabolism.[2]

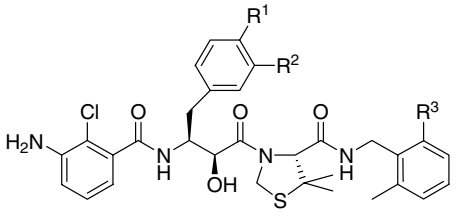
We have previously reported that JE-2147 (**1**, Fig. 1) represents a class of transition-state mimetic dipeptide HIV protease inhibitors containing allophenylnorstatine (Apns: (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid) with a hydroxymethylcarbonyl (HMC) isostere as the active moiety.[3] JE-2147 completely suppresses HIV-1 and HIV-2 strains as well as clinical HIV-1 variants that are highly resistant to marketed protease inhibitors.[4] Although the strong anti-HIV efficacy and safety of JE-2147 were proven in a pilot clinical study, the pharmacokinetic profile of JE-2147 is still not satisfying.[5] In vitro studies have shown that the main metabolites of JE-2147 produced by human hepatocytes are the phenol glucuronide **M1** and the thiazolidine sulfoxide **M2** (Fig. 2) and that JE-2147 is highly metabolized by human liver microsomes in the presence of uridine 5′-diphosphoglucuronic acid (UDPGA), a co-factor of UDP-glucuronosyltransferase (UGT). These findings suggest that co-administration of JE-2147 with ritonavir cannot improve the pharmacokinetic profile of JE-2147 in human, because ritonavir cannot inhibit UGT activity. In a previous paper, we have shown that compounds having a *para* substitution at the phenyl ring of Apns and/or 2, 6-disubstitution at the P2′ benzylamine are

Keywords: HIV; Protease; Inhibitor; SM-309515.
* Corresponding author. Tel.: +81 6 6337 5934; fax: +81 6 6337 6010; e-mail: tsutomu-mimoto@ds-pharma.co.jp

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.10.062



JE-2147 (**1**)



JE-4181 (**2**) : $R^1 = R^2 = R^3 = H$
SM-309515 (**6f**) : $R^1 = H$, $R^2 = OMe$, $R^3 = Me$

Figure 1. Structures of JE-2147 (**1**), JE-4181(**2**), and SM-309515 (**6f**).

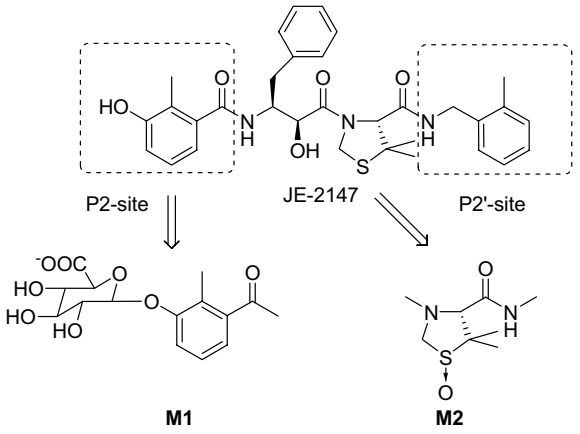


Figure 2. Main metabolites of JE-2147 generated by human hepatocytes.

not subjected to the P2 phenol glucuronidation that occurs with JE-2147.[6] In this communication, we report another strategy to improve the pharmacokinetic profile of our HMC-based HIV protease inhibitors. Ultimately, we found SM-309515 (**6f**) to display a desirable pharmacokinetic profile and a strong antiviral activity.

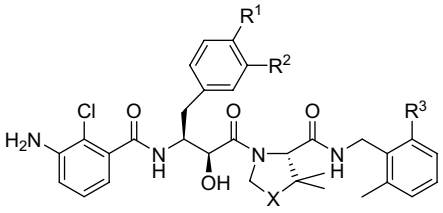
2. Concept of drug design

Information on the interaction between JE-2147 and HIV-1 protease based on X-ray crystallography of their complex[4] provided the basis for the design of novel HIV protease inhibitors that are effective against PI-resistant HIV-1. X-ray crystallography revealed that the hydroxymethylcarbonyl group of Apns (P1) interacts with the aspartic acid carbonyl group of HIV-1 protease active site in essentially the same hydrogen-bonding mode as in the transition state.[4,7] This favorable interaction with the active center of HIV protease contributes to the high activity of JE-2147 against mutant proteases, because the sequence Asp-Thr-Gly in the active center of HIV protease is conserved in any HIV-1 mutant. Another structural feature of JE-2147 is its flexible P2′ benzyl moiety, which can adapt to structural changes in mutant enzymes.[4] Putting this information together, we were able to find JE-4181 (**2**, Fig. 1) as a new HIV protease inhibitor that resists not only glucuronidation but also CYP oxidation by human liver microsomes in vitro (Table 1). JE-4181 also shows good pharmacokinetic profile in rats ($F = 51\%$, data not shown). Although inhibitory activity of JE-4181 against HIV-1 protease is moderate ($K_i = 353$ pM: approximately 10 times lower than that of JE-2147, Table 2), its pharmacokinetic profile makes it a suitable lead compound. In a previous study, we found that substitution at the *meta* or *para* position of the P1 phenyl ring of JE-2147 does not affect this compound's inhibitory activity against HIV-1 protease and that lower-alkoxy substituted compounds show enhanced antiviral activity due to their superior permeation across cell membrane.[6] These findings suggest that introduction of a lower alkoxy group into the P1 phenyl ring of the lead JE-4181 might enhance this compound's antiviral activity, while maintaining its pharmacokinetic advantage.

3. Chemistry

Boc-protected Apns derivatives, the key intermediates of HMC type compounds, were prepared from the corresponding L-phenylalanines as described previously.[8] Hayashi et al. reported that coupling of *N*-protected Apns derivatives to 5,5-dimethyl-1,3-thiazolidine-4-carboxamides often produces low yields due to the formation of Boc-Apns-homobislactone.[9] In our synthesis of JE-2147, the formation of this type of amide bond was promoted by the use of *N*,*N*′-dicyclohexylcarbodiimide (DCC)-1-hydroxybenzotriazole (HOBt) in ethyl acetate as a solvent.[6] The thiazolidine dipeptide amine derivatives **5a**–**h** were prepared by coupling Boc-Apns derivatives to the 5,5-dimethyl-1,3-thiazolidine-4-carboxamides **4a**, **b**, followed by removal of the Boc group under acidic conditions (Scheme 1). The (*RS*)-*N*-*tert*-butoxycarbonyl-3,3-dimethylpyrrolidine-2-carboxylic acid **7**, an intermediate of the pyrrolidine type compounds, was prepared using a procedure described in the literature.[10] The carboxyl group was coupled to 2,6-dimethylbenzylamine by mixed anhydration with diphenylphosphoryl chloridate (DPP-Cl). After removal of the Boc group, Boc-Apns derivatives were coupled by use of 1-ethyl-3-(dimethylaminopropyl)carbodiimide (EDC)-HOBt in *N*,*N*-dimethylformamide (DMF). Pyrrolidine dipeptides in the diastereomixture were separated by silica gel chromatography after removal of the Boc group, and the less polar diastereoisomers of the pyrrolidine dipeptide amine derivatives **9a**–**c** were forwarded to the final step.[11] Coupling of 3-amino-2-chlorobenzoic acid to the dipeptide amine derivatives **5a**–**h** and **9a**–**c** by use

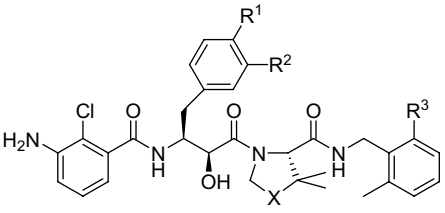
Table 1. In vitro metabolism by human liver microsomes



Compound	Structure				Metabolism remaining (%)	
	X	R^1	R^2	R^3	NADPH	UDPGA
2 (JE-4181)	S	H	H	H	46	>95
6a	S	OMe	H	H	60	>95
6b	S	H	OMe	H	66	93
6c	S	OEt	H	H	65	89
6d	S	OCH_2	O	H	74	>95
6e	S	OMe	H	Me	58	>95
6f (SM-309515)	S	H	OMe	Me	64	>95
6g	S	OEt	H	Me	54	>95
6h	S	OCH_2	O	Me	53	>95
10a	CH_2	OMe	H	Me	63	>95
10b	CH_2	H	OMe	Me	83	>95
10c	CH_2	OEt	H	Me	53	>95
1 (JE-2147)					20	55

Data show the mean residual percent of duplicates. A given test drug (5 μM) was incubated with human liver microsomes (0.5 mg/ml) and cofactor (1 mM NADPH or 5 mM UDPGA) at 37 °C for 30 min. Concentration of residual drug was measured by RP-HPLC.

Table 2. Inhibitory activity against HIV-1 protease and antiviral activity



Compound	Structure				HIV-1 PR K_i (pM)	HIV-1 IIIB EC_{50} (nM)	
	X	R^1	R^2	R^3			+50% Human serum
2 (JE-4181)	S	H	H	H	353	77	165
6a	S	OMe	H	H	311	18	88
6b	S	H	OMe	H	255	21	138
6c	S	OEt	H	H	310	6	35
6d	S	OCH_2	O	H	163	20	77
6e	S	OMe	H	Me	138	24	14
6f (SM-309515)	S	H	OMe	Me	134	4	6
6g	S	OEt	H	Me	152	20	66
6h	S	OCH_2	O	Me	70	17	20
10a	CH_2	OMe	H	Me	804	40	80
10b	CH_2	H	OMe	Me	750	36	87
10c	CH_2	OEt	H	Me	861	63	270
1 (JE-2147)					35	26	52
Saquinavir					138	7	50
Ritonavir					98	2	41
Indinavir					739	23	87
Nelfinavir					931	19	293
Amprenavir					359	16	95
Lopinavir					16	8	8
Atazanavir					50	<4	9

Assay for inhibition of HIV-1 protease was performed by measuring fluoresence intensity of the peptide fragment produced from H-Lys-Ala-Arg-Val-Tyr-Phe(4-NO_2)-Glu-Ala-Nle- NH_2 as a substrate in 200 mM MES buffer, pH 5.5, containing 1 M NaCl, 2 mM dithiothreitol, and 2 mM EDTA-2Na, after incubation at 37 °C for 15 min. Evaluation of in vitro antiviral activity of each test compound against wild-type HIV-1 (strain IIIB) was conducted as described previously.[6]

of EDC-HOBt in DMF gave the target compounds **6a**–**h** and **10a**–**c**, respectively (Schemes 1 and 2).

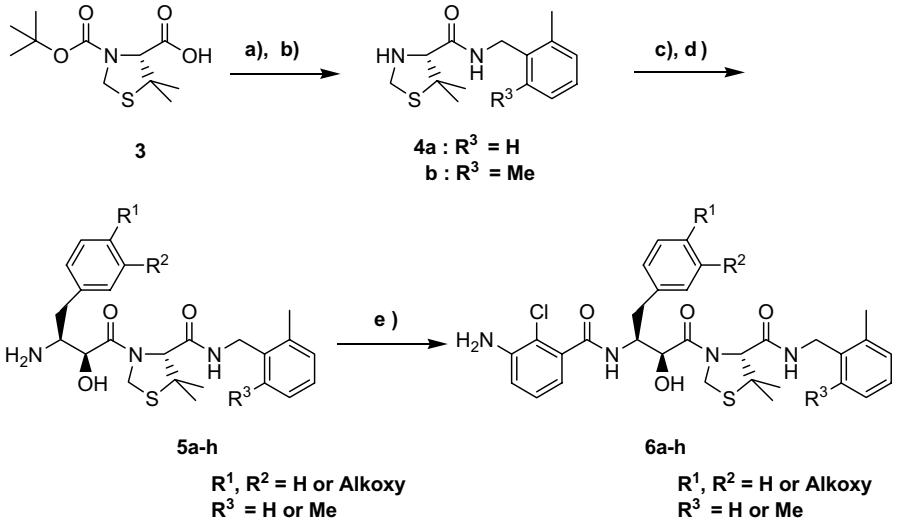
4. Results and discussion

4.1. In vitro metabolism of alkoxy-substituted Apns analogues by human liver microsomes

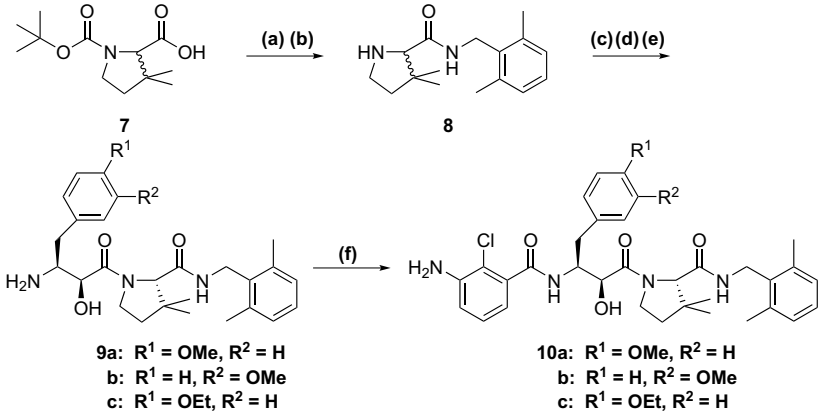
Results of in vitro metabolism of the alkoxy-substituted Apns analogues by human liver microsomes in the presence of UDPGA, a co-factor of UGT, or β-nicotinamide adenine dinucleotide phosphate (NADPH), a co-factor of CYP, are shown in Table 1. All compounds, including the lead JE-4181 (**2**), showed high metabolic resistance to glucuronidation. On the other hand, JE-2147 was strongly glucuronized under our assay conditions. The prepared compounds also showed higher metabolic stability against CYP oxidation than JE-4181. Against our expectation, among the pyrrolidine derivatives **10a**–**c**, in which the thiazolidine was not oxidized, only compound **10b** having 3-OMe substituent on the P1 phenyl group was highly stable against CYP oxidation. This might be due to the unstableness of 4-alkoxy substituent on the P1 phenyl group.

4.2. Inhibitory activity of alkoxy-substituted Apns analogues and other marketed drugs against HIV-1 protease and wild type HIV-1

The inhibitory activity of the prepared HMC-based HIV protease inhibitors as well as that of some marketed drugs against HIV-1 protease is shown in Table 2. The inhibitory activity of JE-4181 against HIV-1 protease was approximately 10 times lower than that of JE-2147 (K_i = 353 and 35 pM, respectively). Substitution by the 4-methoxy group (**6a**), 3-methoxy group (**6b**), 4-ethoxy group (**6c**), or 3,4-methylenedioxy group (**6d**) at the P1 phenyl did not enhance the inhibitory activity of compound **2** against HIV-1 protease (K_i = 163–311 pM). As



Scheme 1. Reagents: (a) Diphenylphosphoryl chloridate, triethylamine, ethyl acetate, and then benzylamines; (b) 4 *N* HCl in ethyl acetate or dioxane; (c) (2*S*,3*S*)-3-(*N*-*tert*-butoxycarbonyl)amino-2-hydroxy-4-arylbutyric acids, DCC, HOBt, ethyl acetate; d) 4 *N* HCl in ethyl acetate or dioxane; (e) 3-amino-2-chlorobenzoic acid, EDC · HCl, HOBt, DMF.



Scheme 2. Reagents: (a) diphenylphosphoryl chloridate, triethylamine, ethyl acetate, and then 2,6-dimethybenzylamine hydrochloride, triethylamine; b) 4 *N* HCl in dioxane, CH_2Cl_2; (c) (2*S*,3*S*)-3-(*N*-*tert*-butoxycarbonyl)amino-2-hydroxy-4-arylbutyric acids, EDC · HCl, HOBt, DMF; (d) 4 *N* HCl in ethyl acetate; (e) separation of diastereomer by silica gel chromatography; (f) 3-amino-2-chlorobenzoic acid, EDC · HCl, HOBt, DMF.

we have shown in previous studies that an additional *ortho* methyl on the P2′ benzyl group of this type of HIV protease inhibitors enhances the inhibitory activity against HIV-1 protease,[3,6] compounds having 2,6-dimethylbenzyl at the P2′ site (**6e**–**h**) were synthesized and their inhibitory activity against HIV-1 protease was evaluated. Compounds **6e**–**h** all showed enhanced HIV-1 protease inhibitory activity. Transformation to the pyrrolidine ring decreased the inhibitory activity against HIV-1 protease with compounds **10a**–**c** showing approximately 5 times lower activity than the corresponding analogues (K_i = 750–861 pM).[12] Although the thiazolidine based compounds **6a**–**h** had less potent HIV protease inhibitory activity than JE-2147, they showed an anti-HIV activity (EC_{50} = 4–24 nM) superior or comparable to that of JE-2147 due to their better cell permeability. We have previously reported that the in vitro antiviral efficacy of JE-2147 is not affected by addition of human serum. This finding is contrary to what has been reported with other HIV protease inhibitors, such as nelfinavir. Unlike most other protease inhibitors, in vitro IC_{50} values of **6e**–**h** did not increase in the presence of 50% human serum and their antiviral activity was comparable to that of lopinavir or atazanavir. Although the pyrrolidine compounds had relatively weak HIV protease inhibitory activity, compounds **10a**, **b** showed antiviral activity in the presence of 50% human serum comparable to that of JE-2147 or other marketed protease inhibitors due to their superior cell permeability.

4.3. Animal pharmacokinetic profile

The pharmacokinetic profiles of compounds **6f**, **10b**, JE-2147, and atazanavir in dogs are summarized in Table 3. Compounds **6f** and **10b**, having 3-methoxy substituent on the P1 phenyl ring and 2, 6-dimethylbenzyl group, had favorable pharmacokinetic profiles exceeding that of JE-2147 or atazanavir (Fig. 3 and Table 3). In addition, plasma concentrations of **6f** and **10b** at 12 h (C_{12h}) after po administration (**6f**: 0.13 μM and **10b**: 0.71 μM) exceeded their antiviral EC_{50}s (measured in the presence of 50% human serum in vitro) by 20- and 8-fold, respectively. These compounds were fully resistant to glucuronidation by human liver microsomes and improved the JE-2147 susceptibility to oxidation by human liver microsomes (Table 4). In addition, **6f** and **10b** showed better stability in human microsomes than in canine microsomes. These findings indicate that **6f** and **10b** would have a better pharmacokinetic profile than JE-2147 or atazanavir in human.

4.4. Co-administration of the synthesized HIV-1 protease inhibitors with ritonavir as pharmacokinetics enhancer

Co-administration of JE-2147 with ritonavir could not be expected to improve the pharmacokinetic profile of JE-2147 in human, because one of the main metabolites of JE-2147 is the phenol glucuronide. Compound **6f**, which showed resistance against glucuronidation, was incubated with human liver microsomes in the absence or presence of ritonavir. Although in the presence of ritonavir the metabolism of **6f** was not effectively inhibited in rat microsomes, it was completely suppressed in canine or human microsomes (Table 5). These findings indicate that pharmacokinetics data collected from the dog are useful to predict the pharmacokinetic profile of **6f** co-administered with ritonavir in humans. In our experiments, concomitant treatment with ritonavir at 7.5 mg/kg considerably enhanced the pharmacokinetics of **6f** (15 mg/kg, po) as indicated by a sixfold increase in AUC (Fig. 3 and Table 3). Plasma drug (**6f**) concentration at 12 h after dosing (C_{12h}) was also enhanced by co-treatment with ritonavir to 2.95 μM, which is over 490 times higher than EC_{50} value in the presence of 50% human serum. As shown in Table 6, **6f** showed antiviral activity against multi-drug-resistant HIV-1s with

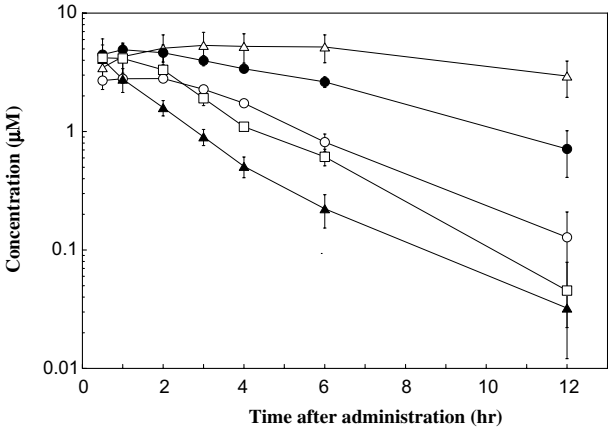


Figure 3. Plasma concentrations of HIV protease inhibitors in Beagle dogs after oral dosing with test compound (15 mg/kg) dissolved in 50% PEG. Each point represents the mean ± SE (n = 3), **6f** (○), **10b** (●), **6f** with ritonavir (7.5 mg/kg po) (△), JE-2147 (▲), and atazanavir (□).

Table 3. Pharmacokinetic parameters of **6f**, **10b**, JE-2147, and atazanavir in beagle dogs[a]

Compound	Route	Dose (mg/kg)	AUC (μM · min)	C_{max} (μM)	T_{max} (min)	V_{dss}[b] (l/kg)	CL[b] (l/h/kg)	F (%)	C_{12h} (μM)
6f (SM-309515)	po	15	936	2.8	120	1.3	1.1	71	0.13
	po[c]	15	3167	5.3	180				2.95
10b	po	15	2203	5.0	60	1.9	1.0	146	0.71
JE-2147	po	15	390	3.1	30	1.6	0.9	25	0.03
atazanavir	po	15	900	4.2	30	2.0	1.6	102	0.05

F (%) was determined by comparing the mean areas under the curve (AUC) after intravenous or oral dosing. C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; V_{dss}, volume of distribution; CL, plasma clearance rate; F (%), percent oral bioavailability; C_{12h}, plasma concentration at 12 h after po administration.

[a] Data are given as mean values.

[b] Calculated from i.v. data.

[c] Co-administered with 7.5 mg/kg of ritonavir (po).

Table 4. Comparison of in vitro metabolism profiles of **6f**, **10b**, JE-2147, and atazanavir

Compound	In vitro metabolism (remaining [%])			
	Dog		Human	
	NADPH	UDPGA	NADPH	UDPGA
6f (SM-309515)	47	>95	64	>95
10b	69	>95	83	>95
JE-2147	49	91	27	42
Atazanavir	85	>95	83	>95

Data show the mean residual of duplicates. Concentration of residual drug was measured by RP-HPLC.

Table 5. In vitro metabolism profile in the presence of ritonavir

RTV conc (μM)	In vitro metabolism (remaining [%])								
	Rat			Dog			Human		
	0	0.5	2.5	0	0.5	2.5	0	0.5	2.5
6f (SM-309515)	21	41	55	36	90	>95	49	93	>95
JE-2147	12	16	31	46	92	93	13	56	59
Lopinavir	11	83	>95	16	83	>95	10	>95	>95

Data show the mean residual of duplicates. Drug (5 μM) with or without ritonavir (RTV) was incubated with liver microsomes (0.5 mg/ml) and cofactors (1 mMNADPH, 5 mM UDPGA) at 37 °C for 30 min. Concentration of residual drug was measured by RP-HPLC.

IC_{50} values ranging from 0.022 to 0.214 μM, and the fold shift of IC_{50} (mutant/wild type) was smaller than that of each marketed protease inhibitor. These findings indicate that **6f**, given in combination with ritonavir, would be useful as salvage therapy for HIV infection.

5. Conclusions

In summary, a series of 3-amino-2-chlorobenzoyl-allophenylnorstatine derivatives, as HIV protease inhibitors, were designed and synthesized. From the structure–activity relationship and metabolism of these compounds, we found that compounds **6f**, **10b**, having 3-methoxy substituent on the P1 phenyl ring and 2,6-dimethylbenzyl group, have a favorable pharmacokinetic profile exceeding that of JE-2147 or atazanavir. Compound **6f** (SM-309515) had more potent inhibitory activity against HIV-1 than JE-2147. From the results of in vitro metabolism of the synthesized compounds using human and canine liver microsomes, SM-309515 is expected to have a good pharmacokinetic profile and anti-HIV activity in humans. Moreover, plasma levels of SM-309515 co-administered with ritonavir exceeded its antiviral EC_{50} against multidrug-resistant HIV-1s. These findings indicate that co-administration of SM-309515 and ritonavir could be useful as salvage therapy for HIV infection.

6. Experimental

6.1. Chemistry general

In general, reagents and solvents were used as purchased without further purification. Column chromatography was performed on an FL60D (Fuji Silysia Chemical LTD). Melting points were measured with a Büchi 535 and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL GSX270 FT NMR spectrometer, and chemical shifts were expressed in δ ppm from the internal standard, tetramethylsilane.

6.1.1. (*R*)-*N*-(2-Methylbenzyl)-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (4a). To a solution of (*R*)-*N*-*tert*-butoxycarbonyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (3, 5.22 g, 20.0 mmol) and triethylamine (3.06 mL, 22.0 mmol) in ethyl acetate (50 mL), DPP-Cl (4.55 mL, 22.0 mmol) was added in an ice-bath, and the mixture was stirred for 1 h. Then to the reaction mixture, 2-methylbenzylamine (2.73 mL, 22.0 mmol) and triethylamine (3.06 mL, 22.0 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, dried over $MgSO_4$, filtered, and concentrated. The residue was redissolved in CH_2Cl_2 (30 mL) and added to 4 N HCl in dioxane (30 mL). After the reaction mixture had been stirred for 2 h, water was added to it; and the 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

Table 6. Sensitivity of mutant HIV-1s to HIV protease inhibitors in Phenosence assay[13]

	EC_{50} (μM)							
	6f	ATV	LPV	AMP	NFV	IDV	RTV	SQV
Wild type	0.0056 (1)	0.0015 (1)	0.0037 (1)	0.014 (1)	0.0054 (1)	0.0076 (1)	0.016 (1)	0.0019 (1)
Strain 1	0.15 (27)	0.092 (62)	0.214 (57)	0.098 (7)	0.452 (82)	0.401 (52)	1.733 (106)	>0.5 (>265)
Strain 2	0.106 (19)	0.048 (32)	0.251 (68)	0.350 (25)	0.346 (64)	0.464 (61)	>3 (>184)	0.097 (51)
Strain 3	0.122 (22)	0.023 (16)	0.657 (176)	0.143 (10)	0.413 (75)	0.753 (97)	>3 (>184)	>0.5 (>265)
Strain 4	0.022 (4)	0.095 (64)	0.066 (18)	0.051 (4)	>1.5 (>273)	0.113 (15)	1.003 (61)	>0.5 (>265)
Strain 5	0.214 (38)	0.266 (180)	0.807 (216)	0.615 (43)	0.776 (141)	>1.5 (>194)	>3 (>184)	>0.5 (>265)
Strain 6	0.096(17)	0.134(90)	0.411 (110)	0.432 (30)	>1.5 (>273)	0.819 (106)	>3 (>184)	0.158 (83)
Strain 7	0.042 (7)	0.074 (50)	0.212 (57)	0.165 (12)	0.515 (94)	0.145 (19)	>3 (>184)	0.141 (75)

Numbers in parentheses represent fold change of EC_{50} values against each isolate compared with EC_{50} values against the wild type. Mutation sites of each strain were as follows: strain l: L10L N37S, M46L, G48V, I54V, Q58E, Q61Q/E, I62I/V, L63P, A71V, V77I, V82A, L90M, I93L; strain 2: L1 0I, G16A, K45V, F53L, I54V, K55N, D60E, L63P, A71V, I72K, V77I, V82F, I84V, L90M, I93L; strain 3: L10I, T12I, E34Q, R41K, M46I, G48V, I50V, F53Y, I54S, I62V, L63P, H69Q, A71V, I72V, V77I, V82A, I93M; strain 4: L10F, K20R, D30N,E35D, M36V, N37T, G51G/E, F53L, I54V, R57K, Q58E, I62V, L63P, A71V, V77I, N88D, L90M; strain 5: L10I, I13V, L33F, R41K, M46L, G48V, F53Y, I54N, Q61E, I62V, L63P, A71V, V77I, V82A, L89V; strain 6: L10I, I13V, K20R, L33F, E35D, M36I, K43T, M46I, I54S, I62V, L63T, I64V, I72V, T74S, V82A, N83D; strain 7: T4A, L10I, K20R, L23I, L33F, E35D, M36I, K43T, M46L.

gave 3.75 g of the title compound with a yield of 71%: mp 77–79 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 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); Anal. Calcd for $C_{14}H_{20}N_2OS$: C, 63.60; H, 7.62; N, 10.60; found: C, 63.53; H, 7.65; N, 10.46.

6.1.2. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(4-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (5a). To a solution of compound **4a** (0.97 g, 9.08 mmol), (2*S*,3*S*)-3-(*N*-*tert*-butoxycarbonyl)-amino-2-hydroxy-4-(4-methoxyphenyl)butyric acid (2.95 g, 9.08 mmol), HOBt (1.23 g, 9.08 mmol) in ethyl acetate (50 mL), and DCC (2.15 g, 10.44 mmol) were added and the mixture was stirred overnight. The reaction mixture was then filtered and the filtrate 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 (10 mL) and added to 4 N HCl in dioxane (10 mL), and the mixture was stirred for 1 h. It was then concentrated under reduced pressure and redissolved in water. The aqueous phase was filtered, and the filtrate was neutralized with 2 N NaOH to give the precipitate. The crude product was recrystallized from ethyl acetate-*n*-hexane to give 4.28 g of the title compound with a yield of 88%: mp 202–204 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.15–1.25 (br, 2H), 1.33 (s, 3H), 1.52 (s, 3H), 2.17 (s, 3H), 2.2–2.3 (m, 1H), 2.64 (t, 1H, J = 8.0 Hz), 3.02 (d, 1H, J = 13.2 Hz), 3.74 (s, 3H), 4.02–4.09 (br, 1H), 4.14 (d, 1H, J = 4.9 Hz), 4.20 (d, 1H, J = 4.9 Hz), 4.36 (s, 1H), 4.90 (s, 2H), 5.30 (d, 1H, J = 7.8 Hz), 6.95 (s, 3H), 6.86 (d, 2H, J = 8.6 Hz), 6.94–7.15 (m, 6H), 8.55 (t, 1H, J = 5.1 Hz); Anal. Calcd for $C_{25}H_{33}N_3O_4S$ 0.25EtOAc: C, 63.26; H, 7.15; N, 8.51; found: C, 63.17; H, 7.18; N, 8.79.

6.1.3. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(4-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6a). To a solution of 3-amino-2-chlorobenzoic acid (70 mg, 0.41 mmol) and **5a** (174 mg, 0.37 mmol) in DMF (4 mL), HOBt (57 mg, 0.42 mmol) and EDCHCl (82 mg, 0.43 mmol) were added in an ice-bath and the mixture was stirred overnight. The reaction mixture was then washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and dried over $MgSO_4$, filtered, and concentrated. The crude product was recrystallized from *n*-hexane-ethyl acetate to give 180 mg of the title compound with a yield of 78%: mp 120–122 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.35 (s, 3H), 1.50 (s, 3H), 2.27 (s, 3H), 2.6–2.8 (m, 2H), 3.70 (s, 3H), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz), 4.2–4.5 (m, 4H), 4.98 (d, 1H, J = 9.2 Hz), 5.12 (d, 1H, J = 8.9 Hz), 5.3–5.5 (m, 3H), 6.37 (d, 1H, J = 6.8 Hz), 6.7–6.9 (m, 3H), 6.98 (t, 1H, J = 7.8 Hz), 7.1–7.2 (br, 1H), 7.2–7.4 (m, 3H), 8.2–8.4 (m, 2H); Anal. Calcd for $C_{32}H_{37}ClN_4O_5S$: C, 61.48; H, 5.97; N, 8.96; found: C, 61.48; H, 6.12; N, 8.72.

6.1.4. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(3-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (5b). Mp 173–175 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.1–1.2 (br, 2H), 1.34 (s, 3H), 1.53 (s, 3H), 2.17 (s, 3H), 2.1–2.3 (m, 1H), 2.6–2.8 (m, 1H), 3.06 (d, 1H, J = 10.5 Hz), 3.75 (s, 3H), 4.03–4.09 (m, 1H), 4.1–4.3 (m, 2H), 4.36 (s, 1H), 4.90 (s, 2H), 5.29 (d, 1H, J = 8.4 Hz), 6.70–6.80 (m, 3H), 6.96 (s, 3H), 7.13–7.23 (m, 2H), 8.53 (t, 1H, J = 5.13 Hz); Anal. Calcd for $C_{25}H_{33}N_3O_4S$: C, 63.67; H, 7.05; N, 8.91; found: C, 63.39; H, 7.13; N, 8.87;

6.1.5. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(3-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6b). Mp 138–140 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.36 (s, 3H), 1.51 (s, 3H), 2.27 (s, 3H), 2.7–2.8 (m, 2H), 3.67 (s, 3H), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz), 4.2–4.5 (m, 4H), 4.99 (d, 1H, J = 9.2 Hz), 5.14 (d, 1H, J = 8.9 Hz), 5.35 (d, 1H, J = 7.0 Hz), 5.41 (s, 2H), 6.35–6.37 (m, 1H), 6.6–6.8 (m, 2H), 6.9–7.0 (m, 3H), 7.1–7.2 (m, 4H), 7.3–7.4 (m, 1H), 8.3–8.4 (m, 2H); Anal. Calcd for $C_{32}H_{37}ClN_4O_5S$ 0.5EtOAc: C, 61.02; H, 6.18; N, 8.37; found: C, 61.09; H, 6.01; N, 8.45.

6.1.6. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(4-ethoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (5c). Mp 208–210 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.1–1.3 (br, 2H), 1.30–1.35 (m, 6H), 1.52 (s, 3H), 2.17 (s, 3H), 2.1–2.3 (m, 1H), 2.63 (t, 1H, J = 8.1 Hz), 3.00 (d, 1H, J = 13.2 Hz), 4.00 (q, 3H, J = 6.8 Hz), 4.1–4.3 (m, 2H), 4.35 (s, 1H), 4.90 (s, 2H), 5.28 (d, 1H, J = 7.8 Hz), 6.84 (d, 2H, J = 8.4 Hz), 6.9–7.1 (m, 5H), 7.1–7.2 (m, 1H), 8.55 (t, 1H, J = 5.1 Hz); Anal. Calcd for $C_{26}H_{35}N_3O_4S$: C, 64.30; H, 7.26; N, 8.65; found: C, 63.98; H, 7.29; N, 8.59.

6.1.7. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(4-ethoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6c). Mp 137–139 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.29 (t, 3H, J = 7.0 Hz), 1.35 (s, 3H), 1.50 (s, 3H), 2.27 (s, 3H), 2.6–2.8 (m, 2H), 3.96 (q, 2H, J = 6.8 Hz), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz), 4.2–4.5 (m, 4H), 4.98 (d, 1H, J = 9.2 Hz), 5.12 (d, 1H, J = 9.5 Hz), 5.3–5.5 (m, 3H), 6.36 (d, 1H, J = 6.2 Hz), 6.7–6.9 (m, 3H), 6.98 (t, 1H, J = 7.8 Hz), 7.1–7.2 (br, 3H), 7.23 (d, 2H, J = 8.9 Hz), 7.3–7.4 (m, 1H), 8.28–8.34 (m, 2H); Anal. Calcd for $C_{33}H_{39}ClN_4O_5S$: C, 62.01; H, 6.15; N, 8.77; found: C, 61.57; H, 6.16; N, 8.63.

6.1.8. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(3,4-methylenedioxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (5d). Mp 202–204 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.1–1.2 (br, 2H), 1.34 (s, 3H), 1.52 (s, 3H), 2.17 (s, 3H), 2.2–2.3 (m, 1H), 2.6–2.8 (m, 1H), 2.98 (d, 1H, J = 11.1 Hz), 4.0–4.1 (m, 1H), 4.1–4.3 (m, 2H), 4.35 (s, 1H), 4.90 (s, 2H), 5.26 (d, 1H, J = 8.1 Hz), 5.97 (s, 2H), 6.59 (d, 1H, J = 7.6 Hz), 6.70 (s, 1H), 6.82 (d, 1H, J = 7.8 Hz), 6.90–7.00 (m, 3H), 7.15–7.17 (m, 1H), 6.70–6.80 (m, 3H), 6.96 (s, 3H), 7.13–7.23 (m, 2H), 8.56 (t, 1H, J = 5.0 Hz); Anal. Calcd for $C_{25}H_{31}N_3O_5S$: C, 61.83; H, 6.43; N, 8.65; found: C, 61.67; H, 6.52; N, 8.63.

6.1.9. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(3,4-methylenedioxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6d). Mp 170–172 °C; ^{1}H NMR (DMSO-d_6) δ

(ppm): 1.36 (s, 3H), 1.51 (s, 3H), 2.27 (s, 3H), 2.6–2.8 (m, 2H), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz), 4.2–4.5 (m, 4H), 4.98 (d, 1H, J = 8.6 Hz), 5.14 (d, 1H, J = 8.9 Hz), 5.32 (d, 1H, J = 6.8 Hz), 5.42 (s, 2H), 5.93 (s, 2H), 6.38 (d, 1H, J = 7.6 Hz), 6.7–6.8 (m, 3H), 6.9–7.0 (m, 2H), 7.1–7.2 (m, 4H), 7.3–7.4 (m, 2H), 8.3–8.4 (m, 2H); Anal. Calcd for $C_{32}H_{35}ClN_4O_6S$: C, 60.13; H, 5.52; N, 8.77; found: C, 59.99; H, 5.42; N, 8.67.

6.1.10. (*R*)-*N*-(2,6-Dimethylbenzyl)-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (4b). To a solution of (*R*)-*N*-*tert*-butoxycarbonyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (**3**, 15.7 g, 60 mmol) and triethylamine (8.76 mL, 63 mmol) in ethyl acetate (230 mL), DPP-Cl (13.0 mL, 63 mmol) was added in an ice-bath; and the mixture was stirred for 4 h. Then to the reaction mixture, 2,6-dimethylbenzylamine hydrochloride (12.1 g, 63 mmol) and triethylamine (18.3 mL, 132 mmol) were added in an ice-bath, followed by stirring 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 (100 mL) and added to 4 N HCl in dioxane (100 mL), and this mixture was stirred for 2 h. After the reaction mixture had been concentrated, the residue was redissolved in CH_2Cl_2-H_2O and neutralized with 2 N NaOH. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a crude product. Recrystallization from *n*-hexane gave 15.7 g of the title compound with a yield of 94%: mp 97–99 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.15 (s, 3H), 1.45 (s, 3H), 2.32 (s, 6H), 3.23 (s, 1H), 3.69 (br, 1H), 3.99 (d, 1H, J = 8.9 Hz), 4.20–4.39 (m, 3H), 7.01–7.13 (m, 3H), 7.99 (br, 1H); Anal. Calcd for $C_{15}H_{22}N_2OS$: C, 64.71; H, 7.96; N, 10.06; found: C, 64.4; H, 8.05; N, 10.04.

6.1.11. (*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(4-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (5e). To a solution of compound **4b** (153 mg, 0.55 mmol), (2*S*,3*S*)-3-(*N*-*tert*-butoxycarbonyl)amino-2-hydroxy-4-(4-methoxyphenyl)butyric acid (163 mg, 0.50 mmol), HOBt (68 mg, 0.50 mmol) in ethyl acetate (5 mL), and DCC (113 mg, 0.55 mmol) were added; and the mixture was stirred overnight. The reaction mixture was then filtered, and the filtrate 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 ethyl acetate (5 mL) and added to 4 N HCl in ethyl acetate (5 mL), and the mixture stirred for 1 h. The reaction mixture was then concentrated under reduced pressure and then redissolved in water. The aqueous phase was filtered, and the filtrate was neutralized with 2 N NaOH to give the precipitate. The crude product was recrystallized from ethyl acetate to give 129 mg of the title compound with a yield of 53%: mp 199–201 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.6–0.8 (br, 2H), 1.34 (s, 3H), 1.52 (s, 3H), 2.09 (s, 6H), 2.0–2.1 (m, 1H), 2.3–2.5 (m, 1H), 3.02 (d, 1H, J = 11.3 Hz), 3.79 (s, 3H), 3.95–4.01 (br, 1H), 4.10 (br, 2H), 4.27 (s, 1H), 4.84 (s, 2H), 5.22 (d, 1H, J = 8.1 Hz), 6.66–6.77 (m, 3H), 6.90 (d, 2H, J = 8.6 Hz), 6.99 (d, 2H, J = 8.4 Hz), 8.17 (br, 1H); Anal. Calcd for $C_{26}H_{35}N_3O_4S$ H_2O: C, 61.60; H, 7.08; N, 8.20; C, 62.00; H, 7.40; N, 8.34.

6.1.12. (*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(4-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6e). Mp 175–177 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.36 (s, 3H), 1.46 (s, 3H), 2.32 (s, 6H), 2.6–2.7 (m, 2H), 3.72 (s, 3H), 4.1–4.3 (m, 2H), 4.44–4.54 (m, 3H), 4.96 (d, 1H, J = 9.5 Hz), 5.15 (d, 1H, J = 8.9 Hz), 5.21 (d, 1H, J = 6.8 Hz), 5.40 (s, 2H), 6.38 (d, 1H, J = 7.3 Hz), 6.7–6.9 (m, 3H), 6.9–7.1 (m, 4H), 7.31 (d, 2H, J = 8.4 Hz), 8.10 (m, 1H), 8.39 (d, 1H, J = 8.4 Hz); Anal. Calcd for $C_{33}H_{39}ClN_4O_5S$: C, 62.01; H, 6.15; N, 8.77; found: C, 61.88; H, 6.04; N, 8.59.

6.1.13. (*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(3-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (5f). Mp 185–187 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.65–0.75 (br, 2H), 1.34 (s, 3H), 1.52 (s, 3H), 2.0–2.1 (m, 1H), 2.11 (s, 6H), 2.4–2.5 (m, 1H), 3.07 (d, 1H, J = 11.1 Hz), 3.79 (s, 3H), 3.99 (t, 1H, J = 8.6 Hz), 4.10 (d, 2H, J = 3.2 Hz), 4.27 (s, 1H), 4.84 (s, 2H), 5.24 (d, 1H, J = 8.1 Hz), 6.64–6.75 (m, 5H), 6.83–6.87 (m, 1H), 7.25 (t, 1H, J = 8.1 Hz), 8.15 (br, 1H); Anal. Calcd for $C_{26}H_{35}N_3O_4S$: C, 64.30; H, 7.26; N, 8.65; found: C, 64.04; H, 7.37; N, 8.61.

6.1.14. (*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(3-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (SM-309515, 6f). Mp 183–185 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.36 (s, 3H, Dmt-5-CH_3), 1.46 (s, 3H, Dmt-5-CH_3), 2.32 (s, 6H, benzylamine-CH_3), 2.6–2.7 (m, 2H, Apns-4-CH_2), 3.76 (s, 3H, Apns-OCH_3), 4.19 (dd, 1H, J = 3.0 Hz, 13.5 Hz, benzylamine-CH_2), 4.2–4.4 (m, 1H, Apns-3-CH), 4.44–4.55 (m, 3H, benzylamine-CH_2, Dmt-4-CH, and Apns-2-CH), 4.97 (d, 1H, J = 8.4 Hz, Dmt-2-CH_2), 5.16 (d, 1H, J = 9.5 Hz, Dmt-2-CH_2), 5.22 (d, 1H, J = 6.8 Hz, Apns-2-OH), 5.41 (s, 2H, benzoyl-NH_2), 6.36 (d, 1H, J = 7.3 Hz, aromatic), 6.71–6.80 (m, 2H, aromatic), 6.96–7.18 (m, 7H, aromatic), 8.09–8.11 (br, 1H, benzylamine-NH), 8.45 (d, 1H, J = 8.9 Hz, Apns-NH); Anal. Calcd for $C_{33}H_{39}ClN_4O_5S$: C, 62.01; H, 6.15; N, 8.77; found: C, 61.93; H, 6.04; N, 8.65.

6.1.15. (*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(4-ethoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (5g). Mp 214–216 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.70 (br, 2H), 1.34 (s, 3H), 1.36 (t, 3H, J = 8.1 Hz), 1.52 (s, 3H), 2.12 (s, 6H), 2.0–2.1 (m, 1H), 2.4–2.5 (m, 1H), 3.02 (d, 1H, J = 11.1 Hz), 3.95–4.01 (br, 1H), 4.02–4.08 (br, 2H), 4.10–4.11 (br, 2H), 4.27 (s, 1H), 4.84 (s, 2H), 5.21 (d, 1H, J = 8.1 Hz), 6.67–6.75 (m, 3H), 6.89 (d, 2H, J = 8.6 Hz), 6.97 (d, 2H, J = 8.4 Hz), 8.16 (br, 1H); Anal. Calcd for $C_{27}H_{37}N_3O_4S$: C, 64.90; H, 7.46; N, 8.41; found: C, 64.70; H, 7.50; N, 8.30.

6.1.16. (*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(4-ethoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6g). Mp 120–122 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.29 (t, 3H, J = 7.0 Hz), 1.35 (s, 3H), 1.50 (s, 3H), 2.27 (s, 3H), 2.6–2.8 (m, 2H), 3.96 (q, 2H, J = 6.8 Hz), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz), 4.2–4.5 (m, 4H), 4.98 (d, 1H,

J = 9.2 Hz), 5.12 (d, 1H, J = 9.5 Hz), 5.3–5.5 (m, 3H), 6.36 (d, 1H, J = 6.2 Hz), 6.7–6.9 (m, 3H), 6.98 (t, 1H, J = 7.8 Hz), 7.1–7.2 (br, 3H), 7.23 (d, 2H, J = 8.9 Hz), 7.3–7.4 (m, 1H), 8.28–8.34 (m, 2H); Anal. Calcd for $C_{34}H_{41}ClN_4O_5S$: C, 62.51; H, 6.33; N, 8.58; C, 62.24; H, 6.38; N, 8.26.

6.1.17. (*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(3,4-methylenedioxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (5h). Mp 183–185 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.65–0.75 (br, 2H), 1.34 (s, 3H), 1.52 (s, 3H), 2.0–2.1 (m, 1H), 2.14 (s, 6H), 2.3–2.4 (m, 1H), 3.00 (d, 1H, J = 11.1 Hz), 3.97 (t, 1H, J = 8.2 Hz), 4.11 (br, 2H), 4.27 (s, 1H), 4.83 (s, 2H), 5.20 (d, 1H, J = 8.4 Hz), 6.05 (d, 1H, J = 3.2 Hz), 6.52 (d, 1H, J = 7.8 Hz), 6.62 (s, 1H), 6.70–6.82 (m, 3H), 6.87 (d, 1H, J = 7.8 Hz), 8.20 (br, 1H); Anal. Calcd for $C_{26}H_{33}N_3O_5S$ 0.5H_2O: C, 61.40; H, 6.74; N, 8.26; found: C, 61.26; H, 6.64; N, 8.13.

6.1.18. (*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(3,4-methylenedioxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6h). Mp 158–160 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.36 (s, 3H), 1.46 (s, 3H), 2.32 (s, 6H), 2.6–2.7 (m, 2H), 4.1–4.3 (m, 2H), 4.44–4.54 (m, 3H), 4.95 (d, 1H, J = 8.6 Hz), 5.15–5.19 (m, 2H), 5.41 (s, 2H), 5.94 (s, 2H), 6.37–6.40 (m, 1H), 6.76–6.87 (m, 3H), 6.96–7.09 (m, 5H), 8.12 (br, 1H), 8.42 (d, 1H, J = 8.4 Hz); Anal. Calcd for $C_{33}H_{37}ClN_4O_6S$: C, 60.68; H, 5.71; N, 8.58; found: C, 60.69; H, 5.78; N, 8.61.

6.1.19. (*RS*)-N-(2,6-Dimethylbenzyl)-3,3-dimethylpyrrolidine-2-carboxamide (8). To a solution of (*RS*)-*N*-*tert*-butoxycarbonyl-3,3-dimethylpyrrolidine-2-carboxylic acid (**7**, 1.05 g, 4.3 mmol) and triethylamine (0.72 mL, 5.2 mmol) in ethyl acetate (30 mL), DPP-Cl (0.98 mL, 4.8 mmol) was added in an ice-bath; and the mixture was stirred for 4 h. Then to the reaction mixture, 2,6-dimethylbenzylamine hydrochloride (0.81 g, 4.8 mmol) and triethylamine (1.50 mL, 10.8 mmol) were added in an ice-bath, followed by stirring 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 ethyl acetate (20 mL) and added to 4 N HCl in ethyl acetate (20 mL), and this mixture was stirred for 2 h. The reaction mixture was neutralized with 2 N NaOH. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a crude product. Recrystallization from *n*-heptane gave 0.92 g of the title compound with a yield of 82%: mp 62–64 °C, ^{1}H NMR (DMSO-d_6) δ (ppm): 0.79 (s, 3H), 1.11 (s, 3H), 2.31 (s, 6H), 2.78–2.91 (m, 3H), 3.11 (s, 1H), 4.27 (d, 2H, J = 3.8 Hz), 7.01–7.11 (m, 3H), 7.62 (t, 1H, J = 4.5 Hz). *Anal*. Calcd for $C_{16}H_{24}N_2O$: C, 73.81; H, 9.29; N, 10.76; found: C, 73.47; H, 9.30; N, 10.64.

6.1.20. (*S*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-amino-4-(3-methoxyphenyl)butanoyl]-3,3-dimethylpyrrolidine-2-carboxamide (9b). To a solution of compound **8** (1.37 g, 5.3 mmol) in DMF (10 mL), (2*S*,3*S*)-3-(*N*-*tert*-butoxycarbonyl)amino-2-hydroxy-4-(3-methoxyphenyl)-butyric acid (1.72 g, 5.3 mmol), HOBt (0.71 g, 5.3 mmol), and EDC · HCl (1.11 g, 5.8 mmol) were added in an ice-bath. After stirring for 16 h, the reaction mixture was poured into ethyl acetate, washed sequentially with 3% K_2CO_3, 5% citric acid, and brine, dried over $MgSO_4$, and then concentrated. The residue was redissolved in ethyl acetate (10 mL) and added to 4 N HCl in ethyl acetate (10 mL). After stirring for 2 h, the reaction mixture was neutralized with 2 N NaOH. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a crude product. The crude product was purified by silica gel chromatography, the less polar product having higher *Rf* value in CH_2Cl_2-MeOH TLC system was collected and recrystallized from *n*-hexane-ethyl acetate to give 1.06 g of the title compound with a yield of 86%: mp 153–155 °C, ^{1}H NMR (DMSO-d_6) δ (ppm): 0.95 (s, 3H), 1.09 (s, 3H), 1.6–1.8 (m, 2H), 2.1–2.2 (m, 1H), 2.15 (s, 6H), 2.5–2.6 (m, 3H), 3.03 (d, 1H, J = 11.1 Hz), 3.5–3.6 (m, 1H), 3.7–3.8 (m, 1H), 3.78 (s, 3H), 3.86–3.93 (m, 2H), 4.0–4.3 (m, 2H), 5.03 (d, 1H, J = 8.4 Hz), 6.71–6.83 (m, 6H), 7.23 (t, 1H, J = 8.0 Hz), 8.07 (br, 1H). *Anal*. Calcd for $C_{27}H_{37}N_3O_4$: C, 69.35; H, 7.98; N, 8.99; found: C, 69.18; H, 8.01; N, 8.88.

6.1.21. (*S*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(3-methoxyphenyl)-butanoyl]-3,3-dimethylpyrrolidine-2-carboxamide (10b). To a solution of 3-amino-2-chlorobenzoic acid (390 mg, 2.3 mmol) and **9b** (1.06 g, 2.3 mmol) in DMF (5 mL), HOBt (310 mg, 2.3 mmol) and EDC · HCl (480 mg, 2.5 mmol) were added in an ice-bath; and the mixture was stirred overnight. The reaction mixture was poured into ethyl acetate, washed sequentially with 3% K_2CO_3, 5% citric acid, and brine, and then dried over $MgSO_4$. After filtered, and concentrated, the crude product was purified by silica gel chromatography and then recrystallized from *n*-hexane-ethyl acetate to give 1.06 g of the title compound with a yield of 75% mp 175–177 °C, ^{1}H NMR (DMSO-d_6) δ (ppm): 1.00 (s, 3H), 1.04 (s, 3H), 1.6–1.7 (br, 1H), 1.9–2.1 (br, 1H), 2.31 (s, 6H), 2.6–2.8 (m, 2H), 3.6–3.7 (br, 1H), 3.76 (s, 3H), 4.0–4.2 (m, 3H), 4.2–4.3 (br, 1H), 4.4–4.6 (m, 2H), 4.90 (d, 1H, J = 6.8 Hz), 5.41 (s, 2H), 6.36 (d, 1H, J = 6.2 Hz), 6.71–6.79 (m, 2H), 6.96–7.11 (m, 6H), 7.15 (t, 1H, J = 7.8 Hz), 8.09 (br, 1H), 8.43 (d, 1H, J = 8.1 Hz). *Anal*. Calcd for $C_{34}H_{41}ClN_4O_5$: C, 65.74; H, 6.65; N, 9.02; found: C, 65.46; H, 6.62; N, 8.84.

6.1.22. (*S*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-amino-4-(4-methoxyphenyl)butanoyl]-3,3-dimethylpyrrolidine-2-carboxamide (9a). Mp 186–188 °C, (DMSO-d_6) δ (ppm): 0.95 (s, 3H), 1.08 (s, 3H), 1.6–1.7 (m, 2H), 2.1–2.2 (m, 1H), 2.16 (s, 6H), 2.50–2.53 (m, 3H), 2.98 (d, 1H, J = 11.2 Hz), 3.5–3.6 (m, 1H), 3.7–3.8 (m, 1H), 3.75 (s, 3H), 3.85–3.93 (m, 2H), 4.0–4.3 (m, 2H), 5.00 (d, 1H, J = 8.2 Hz), 6.75–6.85 (m, 3H), 6.89 (d, 2H, J = 8.4 Hz), 7.04 (d, 2H, J = 8.4 Hz), 8.07 (br, 1H). *Anal*. Calcd for $C_{27}H_{37}N_3O_4$: C, 69.35; H, 7.98; N, 8.99; found: C, 69.14; H, 8.00; N, 8.94.

6.1.23. (S)-N-(2,6-Dimethylbenzyl)-3-[(2S,3S)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(4-methoxyphenyl)-butanoyl]-3,3-dimethylpyrrolidine-2-carboxamide (10a). Mp 195–196 °C, ^{1}H NMR (DMSO-d_6) δ (ppm): 1.00 (s,

3H), 1.03 (s, 3H), 1.6–1.7 (br, 1H), 1.9-2.1 (br, 1H), 2.31 (s, 6H), 2.6–2.8 (m, 2H), 3.6–3.7 (br, 1H), 3.72 (s, 3H), 4.0–4.3 (m, 4H), 4.3–4.4 (br, 1H), 4.50 (dd, 1H, J = 6.5 Hz, 13.8 Hz), 4.89 (d, 1H, J = 7.0 Hz), 5.41 (s, 2H), 6.37 (d, 1H, J = 6.2 Hz), 6.80 (t, 3H, J = 8.6 Hz), 6.96–7.11 (m, 4H), 7.32 (d, 2H, J = 8.4 Hz), 8.09 (br, 1H), 8.38 (d, 1H, J = 8.4 Hz). *Anal*. Calcd for $C_{34}H_{41}ClN_4O_5$: C, 65.74; H, 6.65; N, 9.02; found: C, 65.46; H, 6.70; N, 8.77.

6.1.24. (*S*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-amino-4-(4-ethoxyphenyl)butanoyl]-3,3-dimethylpyrrolidine-2-carboxamide (9c). Mp 181–183 °C, ^{1}H NMR (DMSO-d_6) δ (ppm): 0.95 (s, 3H), 1.08 (s, 3H), 1.32 (t, 3H, J = 7.6 Hz), 1.6–1.8 (m, 2H), 2.2–2.2 (m, 1H), 2.16 (s, 6H), 2.5–2.6 (m, 3H), 2.9–3.0 (m, 1H), 3.5–3.6 (m, 1H), 3.7–3.8 (m, 1H), 3.85–3.96 (m, 2H), 4.0–4.3 (m, 4H), 4.99 (d, 1H, J = 8.4 Hz), 6.75–6.83 (m, 3H), 6.87 (d, 2H, J = 8.6 Hz), 7.02 (d, 2H, J = 8.6 Hz), 8.07 (br, 1H). *Anal*. Calcd for $C_{28}H_{39}N_3O_4$: C, 69.83; H, 8.16; N, 8.72; found: C, 69.57; H, 8.19; N, 8.63.

6.1.25. (*S*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-amino-2-chlorobenzoyl)amino-4-(4-ethoxyphenyl)-butanoyl]-3,3-dimethylpyrrolidine-2-carboxamide (10c). Mp 172–174 °C, ^{1}H NMR (DMSO-d_6) δ (ppm): 1.01 (s, 3H), 1.03 (s, 3H), 1.31 (t, 3H, J = 6.9 Hz), 1.6–1.7 (br, 1H), 1.9–2.1 (br, 1H), 2.31 (s, 6H), 2.6–2.8 (m, 2H), 3.5–3.7 (br, 1H), 3.98 (q, 2H, J = 6.9 Hz), 4.0–4.3 (m, 4H), 4.3–4.4 (br, 1H), 4.49 (dd, 1H, J = 6.5 Hz, 13.5 Hz), 4.89 (d, 1H, J = 7.3 Hz), 5.41 (s, 2H), 6.37 (d, 1H, J = 5.7 Hz), 6.76–6.81 (m, 3H), 6.96–7.09 (m, 4H), 7.30 (d, 2H, J = 8.4 Hz), 8.09 (br, 1H), 8.38 (d, 1H, J = 8.4 Hz). *Anal*. Calcd for $C_{35}H_{43}ClN_4O_5$: C, 66.18; H, 6.82;N, 8.82; found: C, 66.00; H, 6.86;N, 8.65.

6.2. Biological evaluations

HIV protease inhibitory activities, metabolic stabilities, plasma concentrations, and pharmacokinetic parameters of inhibitors were determined by the methods reported previously.[6]

Acknowledgments

The authors thank Southern Research Institute and ViroLogic Incorporation for conducting the wild-type HIV inhibition assay and the mutant HIV inhibition assay (Phenosence assay[13]), respectively. We appreciate the assistance of Mr. Shinji Matsumoto, Mr. Yasuki Hiyama, and Ms. Rena Sekine for the chemical synthesis of HIV protease inhibitors, evaluation of compounds metabolism, and determination of HIV-1 protease activity, respectively. The main part of this work was performed when the authors worked for Pharmaceutical & Biotechnology Laboratory, Japan Energy Corporation.

References and notes

1. Gulick, R. M. *Quality Life Res.* **1997**, *6*, 471.
2. Swainston, H. T.; Scott, L. J. *Drugs* **2005**, *65*, 2309.
3. Mimoto, T.; Kato, R.; Takaku, H.; Nojima, S.; Terashima, K.; Misawa, S.; Fukazawa, T.; Ueno, T.; Sato, H.; Shintani, M.; Kiso, Y.; Hayashi, H. *J. Med. Chem.* **1999**, *42*, 1789.
4. Yoshimura, K.; Kato, R.; Yusa, K.; Kavlick, M. F.; Maroun, V.; Nguyen, A.; Mimoto, T.; Ueno, T.; Shintani, M.; Falloon, J.; Masur, H.; Hayashi, H.; Erickson, J.; Mitsuya, H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8675.
5. Unpublished data.
6. Mimoto, T.; Terashima, K.; Nojima, S.; Takaku, H.; Nakayama, M.; Shintani, M.; Yamaoka, T.; Hayashi, H. *Bioorg. Med. Chem.* **2004**, *12*, 281.
7. Balden, E. T.; Bhar, T. N.; Gulnik, S.; Liu, B.; Topol, I. A.; Kiso, Y.; Mimoto, T.; Mitsya, H.; Erickson, J. W. *Structure* **1995**, *3*, 581.
8. (a) Nishizawa, R.; Saino, T.; Takita, T.; Suda, H.; Aoyagi, T. *J. Med. Chem.* **1977**, *20*, 510; (b) Yuan, W.; Munoz, B.; Wong, C. H.; Haeggstrom, J. Z.; Wetterholm, A.; Samuelsson, B. *J. Med. Chem.* **1993**, *36*, 211; (c) Suzuki, T.; Honda, Y.; Izawa, K.; EP 0767168A2., **1997**; (d) Matsumoto, S.; Matsuo, K.; Sugawa, T.; Moroshima, T.; Inoue, K.; WO98/07687, **1998**; (e) Nishiyama, A.; Inoue, K.; WO2000/53575, **2000**.
9. Hayashi, Y.; Kinoshita, Y.; Hidaka, K.; Kiso, A.; Uchibori, H.; Kimura, T.; Kiso, Y. *J. Org. Chem.* **2001**, *66*, 5537.
10. Morgan, B. A.; Schafer, D. J., US4060603, **1977**.
11. The desired *S*-pyrrolidine diastereoisomer was preserved based on the chemical behavior of corresponding *L*-proline and *R*-thiazolidine derivatives. In addition, HIV-1 protease inhibitory activity of the target compounds **10a-c** derived from the less polar diastereoisomers was far superior to that of the polar diastereoisomers.
12. Similar structure–activity relationships were observed in tripeptide type HMC compounds Mimoto, T.; Hattori, N.; Takaku, H.; Kisanuki, S.; Fukazawa, T.; Terashima, K.; Kato, R.; Nojima, S.; Misawa, S.; Ueno, T.; Imai, J.; Enomoto, H.; Tanaka, S.; Sakikawa, H.; Shintani, M.; Hayashi, H.; Kiso, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1310.
13. Petropoulos, C. J.; Parkin, N. T.; Limoli, K. L.; Lie, Y. S.; Wrin, T.; Huang, W.; Tian, H.; Smith, D.; Winslow, G. A.; Capon, D. J.; Whitcomb, J. M. *Antimicrob. Agents Chemother.* **2000**, *44*, 920.