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Structure–activity and structure–metabolism relationships of HIV protease inhibitors containing the 3-hydroxy-2-methylbenzoyl-allophenylnorstatine structure

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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–metabolism relationship of this type of HIV protease inhibitors, the compounds having *para* substitution of the phenyl ring of Apns and/or 2,6-disubstitution of the P2′ benzylamine were found to be able to avoid the P2 phenol glucuronidation that occurs with SM-319777 (formerly named JE-2147, KNI-764); one of the main metabolic pathways of SM-319777. These new analogues, such as SM-322377, had more desirable pharmacokinetic profiles and more potent antiviral activity against not only wild type HIV-1 but also the multi-drug-resistant HIV-1 than SM-319777.

1. Introduction

In the last decade, a number of agents have become available for the treatment of individuals infected with HIV-1, and combination chemotherapy with reverse transcriptase inhibitors and protease inhibitors has been shown to suppress HIV-1 replication to an undetectable level in patients.[1] However, accumulated data indicate that the development of HIV-1 variants with reduced susceptibility to reverse transcriptase inhibitors and protease inhibitors is directly related to clinical deterioration in those individuals receiving such therapy.[2] Thus, the development of novel compounds that are active against multidrug-resistant HIV-1 variants is urgently needed.[3]

Previously, we have reported that SM-319777[4] (Fig. 1) represents a class of dipeptide HIV protease inhibitors containing allophenylnorstatine with a hydroxymethylcarbonyl (HMC) isostere as the transition-state mimetic. SM-319777 completely suppressed all HIV-1 and HIV-2 strains as well as clinical HIV-1 variants that were highly resistant against all currently available protease inhibitors including saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, and lopinavir.[5] As SM-319777 showed a good pharmacokinetic profile in dogs,[4] we decided to choose this compound as a clinical candidate, which should be useful for salvage therapy; and at present SM-319777 has been examined in an early P-II clinical trial stage. Coadministration of each HIV-1 protease inhibitor with ritonavir is well-known to elevate and sustain the plasma drug levels because of the inhibitiory activity of the latter toward cytochrome P450 (CYP) 3A4-mediated metabolism.[6] The coadministration regimen, for example Kaletra (lopinavir plus ritonavir),[7] shows the favorable pharmacokinetic profile and a strong anti-HIV efficacy. The results of in vitro metabolism studies indicated that ritonavir did not give a remarkable improvement of the metabolism of SM-319777 because SM-319777 was found to be metabolized by not only CYP3A4 but also UDP-glucuronosyl transeferase (UGT) 2B7 in human hepatocytes;[8] and the activity of UGT could not be inhibited by ritonavir. These data suggested that the coadministration of SM-319777 with ritonavir could not be expected to give effective improvement of the pharmacokinetic profile of SM-319777 in humans. In the present study, we disclose the results of our continuing efforts to understand the structure–activity and structure–metabolism relationships of the HMC-based HIV protease inhibitors containing the substituted Apns structure. Ultimately we

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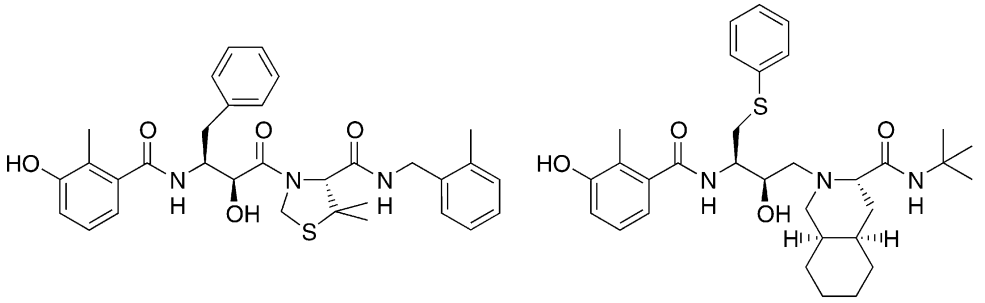


Figure 1. Structures of SM-319777 (left) and nelfinavir (right).

found SM-322377 (**12n**) to display more desirable pharmacokinetic profile and antiviral activity against the multi-drug-resistant HIV-1 than SM-319777.

2. Concept of the drug design

The main metabolites of SM-319777 produced by human hepatocytes in vitro were the phenol glucuronide, **M1**, and the thiazolidine sulfoxide, **M2** (Fig. 2).[8] In the in vitro metabolism study using human liver microsomes, SM-319777 was highly metabolized in the presence of uridine 5′-diphosphoglucuronic acid (UDPGA), a co-factor of UGT (Table 1). On the other hand, nelfinavir[9] (Fig. 1), having the same building block at the P2 site and a phenylthio-structure at the P1 site, was not glucuronized under the same in vitro conditions (Table 1). We speculated that the steric hindrance of the extended aromatic ring system in nelfinavir might be effective to prevent the glucuronidation of the P2 phenol. The X-ray crystallography of HIV protease inhibitors of our Apns series complexed with HIV-1 protease[10,11] showed that the *meta* or *para* position of phenyl moiety of Apns faced the outside of the HIV protease-binding pocket. These data suggested to us that the *meta* or *para* substitution on phenyl moiety of Apns might lead to improvement of the pharmacokinetic property of SM-319777 without changing the binding mode to HIV-1 protease and its excellent viral resistance profile.

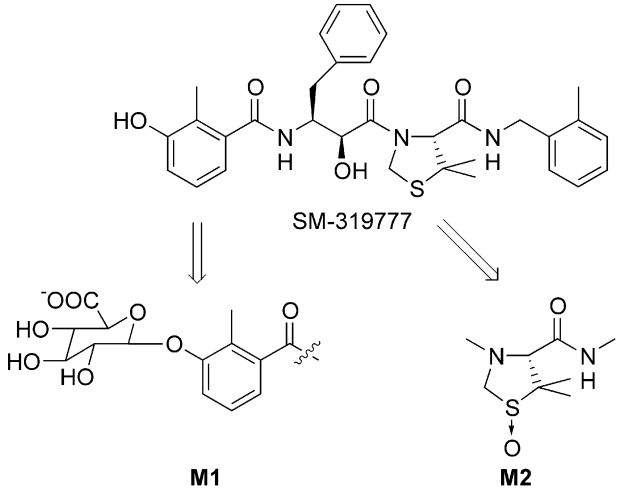
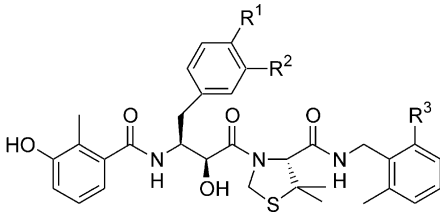


Figure 2. Main metabolites of SM-319777 generated by human hepatocytes.

3. Chemistry

All commercially available optically active amino acids were purchased. The 4-alkoxyphenylalanines were derived from L-tyrosine by alkylation. The other L-phenylalanines were prepared by the acetamidomalonate method or by the Erlenmeyer method, followed by resolution with aminoacylase. Boc-protected (2*S*,3*S*)-3-amino-2-hydroxy-4-arylbutanoic acids, without the 3-methoxy and 3,4-methylenedioxy derivatives, were prepared by the cyanohydrin procedure[12] using aminoaldehydes derived from L-phenylalanines. The Boc-protected (2*RS*,3*S*) mixture thus obtained was converted to the benzyl ester, and the (2*S*,3*S*) enantiomer was easily separated by recrystallization from *n*-hexane/ethyl acetate, and then debenzylation by hydrogenation (H_2–Pd/C) gave the desired Boc-Apns derivatives, (2*S*,3*S*) enantiomers. In the case of the compounds having

Table 1. In vitro metabolism by human liver microsomes



Compd	Structure			Metabolism: remaining (%)	
	R1	R2	R3	UDPGA	NADPH
SM-319777	H	H	H	55	20
12a	OMe	H	H	>95	27
12b	H	OMe	H	63	47
12c	OEt	H	H	91	18
12d	O*n*Pr	H	H	>95	25
12e	O*i*Pr	H	H	>95	13
12f	–O–CH_2–O–		H	>95	62
12g	Me	H	H	>95	43
12h	H	Me	H	68	55
12i	Cl	H	H	>95	41
12j	H	Cl	H	53	43
12k	F	H	H	38	42
12l	H	F	H	71	53
12m	OMe	H	Me	>95	34
12n (SM-322377)	H	OMe	Me	>95	51
12o	–O–CH_2–O–		Me	>95	45
Nelfinavir				>95	52

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. Residual drugs were measured by RP-HPLC.

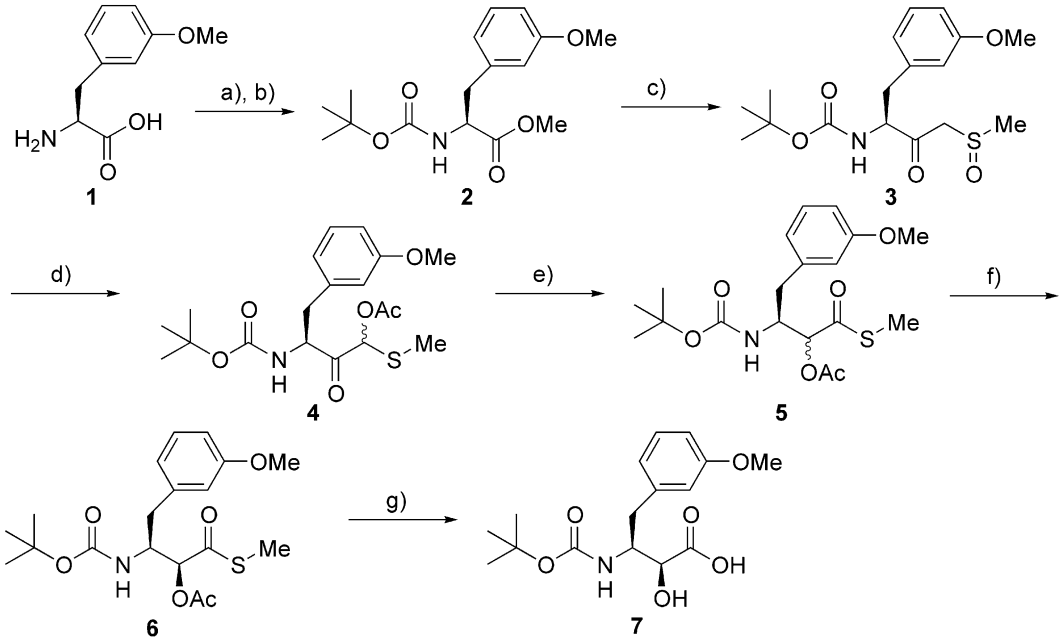
3-methoxy or 3,4-methylenedioxy group, the cyanohydrin method could not give the desired products in satisfactory yield due to their instability under acid hydrolysis condition of cyanohydrin intermediates, so another method reported by Ajinomoto's group was applied to the synthesis of these derivatives (Scheme 1).[13] The reaction of Boc-protected L-3-methoxyphenylalanine methyl ester (**2**) derived from the corresponding amino acid **1** with the carbanion derived from dimethylsulfoxide gave the diastereomixture of β-ketosulfoxide **3**. α-Keto-hemimercaptal acetate **4** was obtained by reacting **3** with acetic anhydride in CH_2Cl_2 in the presence of pyridine (Pummerer rearrangement), and then **4** was rearranged into a β-amino-α-acyloxythioester **5** possibly via 1,4-acyl transfer in enolate form in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene in *N,N*-dimethylformamide (DMF) at −20 °C. The diastereomeric ratio (2*S*/2*R*) of **5** was about 3:2, and the desired (2*S*,3*S*) enantiomer **6** was easily separated by recrystallization from *n*-hexane/ethyl acetate. Saponification of **6** gave the desired Boc-Apns derivative **7**.

Boc-Apns derivatives thus obtained were coupled with previously reported[3] 5,5-dimethyl-1,3-thiazolidine-4-carboxamides **9a**,**b**. Hayashi et al. reported[14] that low yields were often observed in the coupling of *N*-protected Apns derivatives and 5,5-dimethyl-1,3-thiazolidine-4-carboxamides, caused by the formation of Boc-Apns-homobislactone. In their report, 1-ethyl-3-(dimethylaminopropyl)carbodiimide-1-hydroxy-7-azabenzotriazole (HOAt) method in DMF was recommended. In the course of our scale-up synthesis of SM-319777, *N,N*′-dicyclohexylcarbodiimide (DCC)-1-hydroxybenzotriazole (HOBt) method in ethyl acetate was found out to be effective for the formation of this amide bond.[15] The Boc-protected dipeptide amide thus obtained were deprotected under acidic conditions gave the dipetide amine derivatives **10a–o**. The coupling of 3-acetoxy-2-methylbenzoic acid to the dipeptide amine derivatives **10a–o** by the mixed anhydride method using diphenylphosphoryl chloridate gave **11a–o**, which after saponification, gave compounds **12a–o** in good yield (Scheme 2).

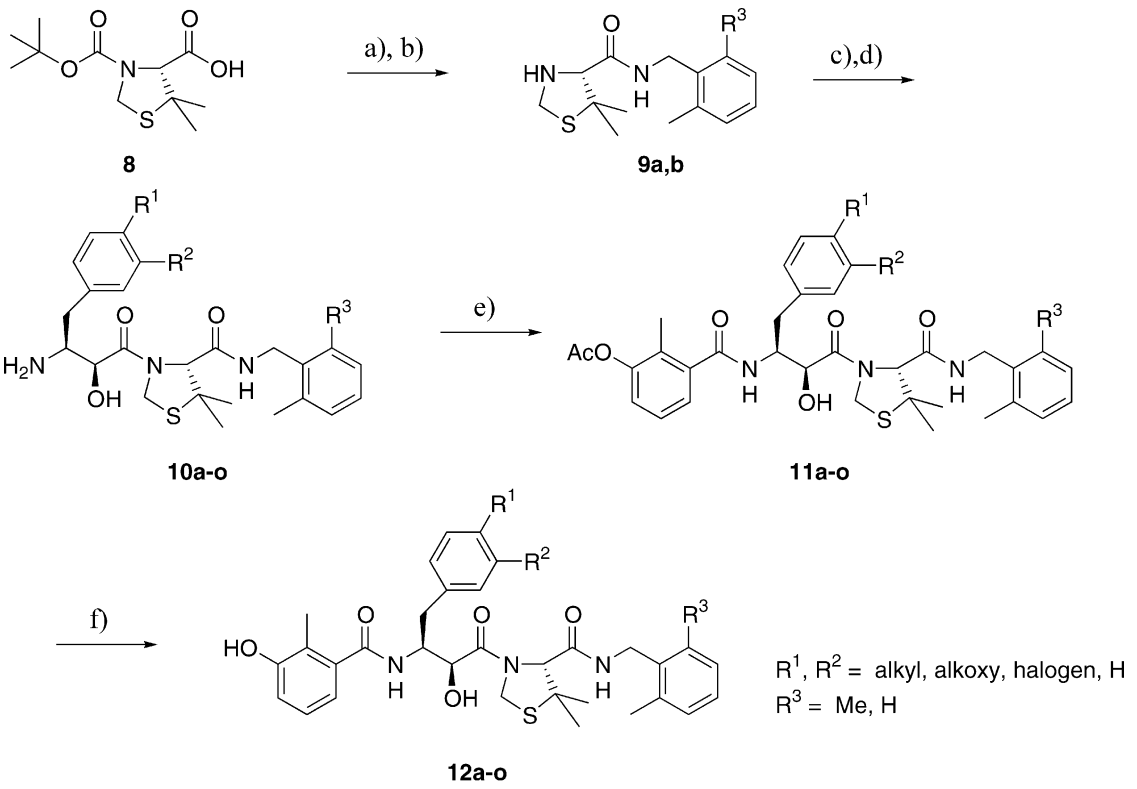
4. Results and discussion

4.1. Structure–metabolism relationship by human liver microsomes in vitro

Table 1 shows the results of our in vitro metabolism study on these compounds. The compounds containing the *para* substituent (R^1) on the phenyl ring, except **12k**, were stable against the glucuronidation; whereas the compounds having the *meta* substitution (R^2), **12b**, **h**, **j**, and **l**, or a small *para* substitution such as fluorine **12k**, on the phenyl ring remained susceptible to the glucuronidation of the P2 phenol. These data suggest that the steric bulk of the *para* substituent of the Apns ring could prevent the glucuronidation of the P2 phenol. Moreover, the introduction of a lower alkoxy substituent to the phenyl ring provided an increase in solubility (data not shown). However, the *para* alkoxy-substituted analogues did not show improved stability against CYP oxidation when their in vitro metabolism was examined with human liver microsomes in the presence of β-nicotinamide adenine dinucleotide phosphate (NADPH). In contrast, the *meta* alkoxy analogues **12b**,**f** were more stable than SM-319777 against the CYP oxidation. In the case of the compounds containing the 2,6-dimethylbenzyl group at the P2′ site (R^3 = Me), the *meta* alkoxy analogues **12n**,**o** also had such stability against the CYP



Scheme 1. (a) di-*tert*-Butyl dicarbonate, triethylamine, THF–H_2O; (b) methyl iodide, K_2CO_3, DMF; (c) DMSO, sodium amide, THF; (d) acetic anhydride, pyridine, 4-(dimethylamino)pyridine, CH_2Cl_2; (e) 1,8-diazabicyclo[5.4.0]undec-7-ene, DMF; (f) recrystalization from *n*-hexane/ethyl acetate; (g) NaOH aq, methanol.



Scheme 2. (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 acid, *N*,*N*′-dicyclohexylcarbodiimide, 1-hydroxybenztriazole, ethyl acetate; (d) 4 N HCl in ethyl acetate (or dioxane); (e) 3-acetoxy-2-methylbenzoic acid, diphenylphosphoryl chloridate, triethylamine, ethyl acetate; (f) NaOH aq, methanol.

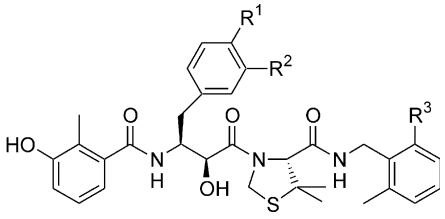
oxidation. Surprisingly, the compound of this class having no *para* substitution, **12n**, was not affected under the in vitro glucuronizing condition. These data suggest that the *para* substitution of Apns or the additional *ortho* methyl group on the P2′ benzyl group of SM-319777 could reduce the affinity of SM-319777 for UGT. Compound **12n** had stability comparable to that of nelfinavir against both CYP oxidation and UDP glucuronidation.

4.2. Structure–activity relationship of HIV-1 protease inhibition and antiviral activity

Table 2 represents the results of the study on HIV-1 protease inhibitory activity[16] and anti-HIV activity of the selected compounds that were stable against the UDP glucuronidation. As the inhibitory activity of SM-319777 toward HIV protease was not affected by the introduction of the *meta*- or *para* subsituent on the phenyl moiety of Apns, the P1 phenyl-substituted compounds, **12a**,**c**–**f**, had activity comparable to that of SM-319777 in terms of HIV-1 protease inhibition (K_i = 31–59 pM). Moreover, the series of compounds containing the additional *ortho* methyl group at the P2′ site (R^3 = Me), **12m–o**, showed slightly more potent activity than SM-319777 (K_i = 22–29 pM).

Although these compounds had nearly the same HIV protease inhibitory activity as SM-319777, cell culture assays under the condition of 15% fetal bovine serum demonstrated that almost all compounds of this study

Table 2. HIV-1 protease (HIV-1 PR) inhibition and anti-HIV activity



Compd	Structure			HIV-1 PR inhibition	HIV-1 IIIB; EC_{50} (nM)	
	R1	R2	R3	K_i (pM)	15% fetal bovine serum	50% human serum
SM-319777	H	H	H	35	26	52
12a	OMe	H	H	31	6.6	28
12c	OEt	H	H	38	6.5	9.7
12d	O*n*Pr	H	H	59	3.2	25
12e	O*i*Pr	H	H	40	7.9	17
12f	–O–CH_2–O–		H	37	4.8	52
12m	OMe	H	Me	29	7.6	14
12n (SM-322377)	H	OMe	Me	29	2.4	9.0
12o	–O–CH_2–O–		Me	22	3.0	14
Nelfinavir				931	19	293

Assay of HIV-1 protease was performed by measuring the fluorescence 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, with incubation at 37 °C for 15 min. Evaluation of in vitro antiviral activities of test compounds against wild-type HIV-1 (strain IIIB) was conducted as described previously[16] (details are written in the Experimental).

were more effective than SM-319777 in suppressing the spread of acute HIV-1 infection, probably due to their superior permeation of the cell membrane. A previous paper reported[4] that the in vitro antiviral efficacy of SM-319777 was not affected by the addition of the human relevant protein, which was different from the case for other HIV protease inhibitors such as nelfinavir and ritonavir. Some compounds in this study showed the anti-HIV activity superior to that of SM-319777 even in the presence of 50% human serum. We confirmed that the substitution of a lower alkoxy group on the Apns ring reduced the protein binding affinity, especially for α-acid glycoprotein.[17] Above all, the compounds containing the 2,6-dimethylbenzyl group at the P2′ site, **12m–o**, which had the lower K_i values, showed extremely potent anti-HIV activity even in the presence of 50% human serum.

4.3. Antiviral activity against multi-drug resistant HIV-1

As shown in Table 3, the indinavir-resistant clinical isolates used in this experiment were also resistant to nelfinavir, amprenavir, and lopinavir. All isolates had a high level of resistance to indinavir (14–82-fold), nelfinavir (147–1029-fold), amprenavir (7–29-fold), and lopinavir (306–527-fold), compared with that against a wild-type clinical isolate. In contrast, SM-319777 suppressed the replication of all isolates, with EC_{50} values of less than 100 nM; less than single figure-fold change in EC_{50} was found compared with the EC_{50} against wild-type HIV-1. Compound **12a**, having a *para* methoxy group at the P1 site of SM-319777, showed nearly the same antiviral property as SM-319777. This result suggests that the introduction of *para* methoxy group did not affect the mode of binding of SM-319777 to HIV-1 protease, and **12a** retained the unique resistance profile of SM-319777. Moreover, SM-322377 (**12n**) had more potent anti-HIV activity than SM-319777 and maintained the same resistance profile as SM-319777; and SM-322377 showed the greatest antiviral activity with EC_{50} values of less than 10 nM against all three mutants tested.

4.4. In vitro human liver microsomal metabolism in the presence of ritonavir and pharmacokinetics of coadministration with ritonavir

According to the in vitro metabolism study on SM-319777 incubated with human liver microsomes, ritonavir (CYP3A4 inhibitor[6]) did not show the inhibitory activity against the metabolism of SM-319777, because the phenol-glucuronidation was one of the main metabolic pathway of SM-319777 and ritonavir could not inhibit UGT enzyme. When compound **12a** or **n**, which had resistance against the glucuronidation, was incubated with human or canine liver microsomes in the absence or presence of ritonavir (Table 4), the metabolism of these compounds was effectively inhibited by the addition of 2.5 μM ritonavir, however the metabolism of **12a**,**n**, and SM-319777 was not effectively inhibited in rat microsomes. These data indicate that the information from canine microsomes is a good predictor of the pharmacokinetic profiles of **12a,** and **12n** coadministered with ritonavir in humans.

Table 3. Sensitivity of mutant HIV-1s to HIV protease inhibitors

Compd	HIV-1/PBMC; EC_{50} (nM)							
	Wild type		1002-60		1026-40		052-52	
SM-319777	17	(1)	37	(2)	103	(6)	17	(1)
12a	6.6	(1)	17	(3)	99	(15)	33	(5)
12n (SM-322377)	1.0	(1)	4.5	(5)	8.9	(9)	<1	(1)
Indinavir	12	(1)	985	(82)	450	(38)	169	(14)
Nelfinavir	1.2	(1)	1235	(1029)	335	(279)	176	(147)
Amprenavir	26	(1)	194	(7)	752	(29)	535	(21)
Lopinavir	1.3	(1)	573	(441)	398	(306)	685	(527)

Evaluation of in vitro antiviral activities of test compounds against wild-type HIV-1 (strain IIIB) and drug-resistant HIV containing multi-mutations isolated from patients conferring to indinavir were conducted as described previously[18] (details are written in the Experimental). The mutation sites of each strain were as follow: 1002-60: L10I/M46I/I54V/L63P/V82F/L90M; 1026-40: L10I/M46I/I54V/L63P/A71V/V82A/L90M; 052-52: L10R/M46I/L63P/A71V/V82T/I84V. Numbers in parentheses represent fold changes of EC_{50} values against each isolate compared with EC_{50} values against the wild type.

Table 4. In vitro metabolism by liver microsomes in the presence of ritonavir

Compd	Human		Rat		Dog	
	(−) RTV	(+) RTV	(−) RTV	(+) RTV	(−) RTV	(+) RTV
SM-319777	13	59	12	31	46	>95
12a	32	89	23	69	38	>95
12n (SM-322377)	46	87	9	34	53	>95
Lopinavir	10	>95	11	>95	16	>95

Data show the mean residual percent of duplicates. Drug (5 μM) with or without 2.5 μM ritonavir (RTV) were incubated with liver microsomes (0.5 mg/mL) and cofactors (1 mM NADPH, 5 mM UDPGA) at 37 °C for 30 min. Residual drug was measured by RP-HPLC.

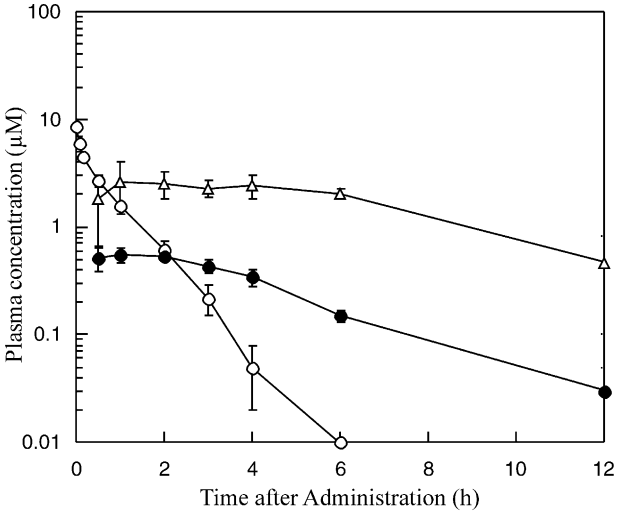


Figure 3. Plasma concentrations of SM-322377 (**12n**) after intravenous or oral administration to beagle dogs. SM-322377 was dissolved in 50% PEG. The plasma concentration was measured by HPLC method as described in the Experimental. Each point represents the mean ± SE of three dogs. ○, 5 mg/kg intravenous injection; ●, 15 mg/kg oral administration; △, 15 mg/kg oral administration with 7.5 mg of ritonavir.

Table 5. Pharmacokinetic parameters of SM-322377 (**12n**) in beagle dogs

Route	Dose (mg/kg)		AUC (μM*h)	C_{max} (μM)	T_{max} (h)	$T_{1/2}$ (h)	V_{dss} (l/kg)	CL (l/h/kg)	F (%)
	12n (SM-322377)	Ritonavir							
iv	5	0	5.0			0.5	1.4	1.6	
po	15	0	3.0	0.6	1.0				20
po	15	7.5	23.7	2.7	1.0				

Data are given as mean values of triplicates. F(%) was determined by comparing the mean areas under the curves (AUC) after intravenous and oral doses. CL, plasma clearance rate; V_{dss}, volume of distribution; $T_{1/2}$, half-life in plasma; C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; F(%), percent orally bioavailable.

Compound **12n** was orally administered to dogs at 15 mg/kg with or without 7.5 mg ritonavir/kg. Plasma concentrations are shown in Figure 3, and pharmacokinetic parameters are given in Table 5. The concentration of **12n** in the plasma was enhanced by coadministration with ritonavir, because CYP3A4, the major metabolic enzyme of **12n**, was inhibited by ritonavir. The value for the area under the plasma concentration curve (AUC) was enhanced by about 8-fold, and the coadministration of **12n** with ritonavir provided a plasma concentration of **12n** that exceeded the antiviral EC_{50} (measured in vitro in the presence of 50% human serum) by 30-fold for at least 12 h. These data suggest that SM-322377 (**12n**) coadministered with ritonavir can be expected to show a favorable pharmacokinetic profile and the strong anti-HIV efficacy in human use.

5. Conclusions

In summary, we designed and synthesized a series of the substituted-Apns-containing HIV protease inhibitors. From the structure-metabolism relationships studies on these types of HIV protease inhibitior, we found out that the *para* substitution of Apns phenyl ring and/or the 2, 6-disubstitution of the P2′ benzylamine could prevent the P2 phenol glucuronidation by human liver microsomes, which gives rise to one of the main metabolites of SM-319777 by human hepatocytes. In the in vitro metabolism by human liver microsomes, the metabolism of **12a**,**n** was effectively inhibited by the addition of ritonavir. The plasma levels of SM-322377 (**12n**) coadministered with ritonavir exceeded the antiviral EC_{50} (measured in vitro in the presence of 50% human serum) by 30-fold for at least 12 h. As these compounds maintained a potent antiviral activity against the multidrug resistant HIV-1, the coadministration regimen with ritonavir should be useful for salvage therapy.

6. Experimental

6.1. Chemistry general

In general, reagents and solvents were used as purchased without further purification. Column chromatography was performed on FL60D (Fuji Silysia Chemical Ltd). Melting points were measured with a Büchi 535 melting point apparatus and 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. (2*S*,3*S*)-2-Acetoxy-3-(*N*-*tert*-butoxycarbonyl) amino-4-(3-methoxyphenyl)butyric acid methylthioester (6). Sodium amide (3.43 g, 88.0 mmol) was suspended in tetrahydrofuran (THF, 60 mL) and DMSO (10.4 mL, 147 mmol), and heated at 50 °C for 3 h. After cooling, a THF (30 mL) solution of *N*-*tert*-butoxycarbonyl-(L)-3-methoxyphenylalanine methyl ester (9.05 g, 29.3 mmol) was added and stirred at 0 °C for 2 h. The reaction mixture was extracted with 1 N HCl (100 mL) and ethyl acetate (100 mL) and the organic layer was washed with 50 mL of brine, dried over $MgSO_4$, and concentrated. The resulting residue was purified by silica-gel chromatography to obtain 6.15 g of the crude product **3** as an oil (yield 59%). Compound **3** (6.15 g, 17.3 mmol) was dissolved in CH_2Cl_2 (160 mL), pyridine (16.3 mL), and acetic anhydride (16.3 mL, 173 mmol), and then 4-(dimethylamino)pyridine (104 mg, 0.87 mmol) was added, and the mixture was stirred at 25 °C for 16 h. To the reaction mixture was added 200 mL of water, with stirring for 1 h. The organic layer was washed with 1 N HCl, 5% $NaHCO_3$, and brine, and then dried over Na_2SO_4 and concentrated. The resulting residue was purified by silica-gel chromatography to obtain 5.19 g of the crude compound **4**. Compound **4** (5.19 g, 13.1 mmol) was dissolved in DMF (25 mL) and cooled to −30 °C, and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.99 mL, 13.1 mmol) was then added thereto. Having been stirred at −10 °C for 3 h, this mixture was partitioned between ethyl acetate and 1 N HCl. The organic layer was washed with 1 N HCl, 5% $NaHCO_3$, and 5% NaCl, dried over $MgSO_4$, and then concentrated. The residue thus obtained was recrystallized from *n*-hexane (three times), giving 1.70 g of the title compound in 25% yield (total, 15%): mp 114–116 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.29 (s, 9H), 2.21 (s, 3H), 2.26 (s, 3H), 2.6–2.7 (m, 2H), 3.72 (s, 3H), 4.09–4.19 (m, 1H), 5.31 (d, 1H, J = 4.3 Hz), 6.73–6.78 (m, 3H), 7.13–7.20 (m, 2H). Anal. calcd for $C_{19}H_{27}NO_6S$: C, 57.41, H, 6.85, N, 3.52; Found C, 57.49; H, 6.84; N, 3.36.

6.1.2. (2*S*,3*S*) - 3 - (*N* - *tert* - butoxycarbonyl)amino - 2-hydroxy-4-(3-methoxyphenyl)butyric acid (7). Compound **6** (1.65 g, 4.16 mmol) was dissolved in methanol (30 mL), and 1.5 N NaOH (11 mL) was added, and the mixture was stirred at room temperature overnight. The reaction mixture was partitioned between ethyl

acetate and 1 N HCl, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. Recrystallization from *n*-hexane/ethyl acetate gave 1.19 g of the title compound, in 88% yield: mp 121–123 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.27 (s, 9H), 2.63–2.69 (m, 2H), 3.72 (s, 3H), 3.85–3.99 (m, 2H), 6.69–6.75 (m, 4), 7.15 (t, 1H, J = 6.8 Hz). Anal. calcd for $C_{16}H_{23}NO_6$: C, 59.06; H, 7.13; N, 4.31; Found C, 59.01; H, 7.14; N, 4.19.

6.1.3. (*R*)-*N*-(2-Methylbenzyl)-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (9a). To a solution of (*R*)-*N*-*tert*-butoxycarbonyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (5.22 g, 20.0 mmol) and triethylamine (3.06 mL, 22.0 mmol) in ethyl acetate (50 mL), diphenylphosphoryl chloridate (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 the mixture was stirred overnight. The reaction mixture was washed sequentially with 1 N HCl, 3% K_2CO_3, and brine; dried over $MgSO_4$, filtered, and concentrated. The residue was redissolved in CH_2Cl_2 (30 mL) and stirred for 2 h with 4 N HCl in dioxane (30 mL). To the reaction mixture, water was added, and aqueous phase was washed with toluene, neutralized with 2 N NaOH, and extracted with ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a crude product. Recrystallization from *n*-hexane/ethyl acetate gave 3.75 g of the title compound, 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.

6.1.4. (*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 (10a). To a solution of compound **9a** (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), and 1-hydroxybenztriazole (1.23 g, 9.08 mmol) in ethyl acetate (50 mL), *N*,*N*′-dicyclohexylcarbodiimide (2.15 g, 10.44 mmol) was added, and the mixture was stirred overnight. The reaction mixture was 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 stirred for 1 h with 4 N HCl in dioxane (10 mL). The reaction mixture was 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 a precipitate. The crude product was recrystallized from ethyl acetate–*n*-hexane to provide 4.28 g of the title compound, in 88% yield: 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.

6.1.5. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(4-methoxyphenyl)-butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12a). To a solution of 3-acetoxy-2-methylbenzoic acid (79 mg, 0.41 mmol) and triethylamine (57 μL, 0.41 mmol) in ethyl acetate (2 mL), diphenylphosphoryl chloridate (84 μL, 0.41 mmol) was added in an ice-bath, and the reaction mixture was stirred for 1 h. Then to the reaction mixture, compound **10a** (174 mg, 0.37 mmol), and triethylamine (62 μL, 0.45 mmol) were added in an ice-bath followed by stirring overnight. The reaction mixture was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and then concentrated. To the solution of the resulting residue in methanol (1.5 mL), 1 N NaOH (0.75 mL, 0.75 mmol) was added; and the mixture was stirred for 1 h. The mixture was acidified with 1 N HCl, and the aqueous phase was extracted with ethyl acetate. The organic extract was washed sequentially with 1 N HCl, 3% K_2CO_3, and brine; dried over $MgSO_4$; filtered; and concentrated. The crude product, recrystallized from *n*-hexane/ethyl acetate, gave 176 mg of the title compound, with a yield of 79%: mp 147–150 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.83 (s, 3H, benzoyl-CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.6–2.8 (m, 2H, Apns-4-CH_2), 3.70 (s, 3H, Apns-OCH_3), 4.09 (dd, 1H, J = 5.1 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.5 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.01 (d, 1H, J = 9.5 Hz, Dmt-2-CH_2), 5.13 (d, 1H, J = 8.6 Hz, Dmt-2-CH_2), 5.45 (d, 1H, J = 6.8 Hz, Apns-2-OH), 6.58 (d, 1H, J = 7.0 Hz, aromatic), 6.7–6.9 (m, 3H, aromatic), 6.96 (t, 1H, J = 7.8 Hz, aromatic), 7.0–7.4 (m, 6H, aromatic), 8.11 (d, 1H, J = 8.1 Hz, Apns-NH), 8.32 (t, 1H, J = 5.4 Hz, benzylamine-NH), 9.40 (s, 1H, benzoyl-OH). Anal. calcd for $C_{33}H_{39}N_3O_6$: C, 65.43; H, 6.49; N, 6.94; Found C, 65.28; H, 6.52; N, 6.88.

6.1.6. (*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 (10b). 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.

6.1.7. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(3-methoxyphenyl)-butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12b). Mp 132–135 °C, ^{1}H NMR (DMSO-d_6): δ (ppm); 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.86 (s, 3H, benzoyl-CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.6–2.8 (m, 2H, Apns-4-CH_2), 3.68 (s, 3H, Apns-OCH_3), 4.12 (dd, 1H, J = 4.3 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.5 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.01 (d, 1H, J = 9.2 Hz,

Dmt-2-CH_2), 5.15 (d, 1H, J = 8.6 Hz, Dmt-2-CH_2), 5.41 (d, 1H, J = 6.8 Hz, Apns-2-OH), 6.57 (d, 1H, J = 7.3 Hz, aromatic), 6.7–6.8 (m, 2H, aromatic), 6.9–7.0 (m, 3H, aromatic), 7.1–7.2 (m, 4H, aromatic), 7.3–7.4 (m, 1H, aromatic), 8.18 (d, 1H, J = 8.1 Hz, Apns-NH), 8.36 (t, 1H, J = 5.4 Hz, benzylamine-NH), 9.40 (s, 1H, benzoyl-OH). Anal. calcd for $C_{33}H_{39}N_3O_6S$ 0.5EtOAc: C, 64.69; H, 6.67; N, 6.47; Found C, 64.71; H, 6.62; N, 6.81.

6.1.8. (*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 (10c). 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.

6.1.9. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(4-ethoxyphenyl)-butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12c). Mp 137–139 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.29 (t, 3H, J = 7.0 Hz, OEt–CH_3), 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.86 (s, 3H, benzoyl-CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.6–2.8 (m, 2H, Apns-4-CH_2), 3.96 (q, 2H, J = 6.9 Hz, Apns-OCH_2), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz, benzylamine-CH_2), 4.2–4.5 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.00 (d, 1H, J = 9.5 Hz, Dmt-2-CH_2), 5.13 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.43 (d, 1H, J = 7.0 Hz, Apns-2-OH), 6.57 (d, 1H, J = 6.8 Hz, aromatic), 6.78 (d, 2H, J = 8.6 Hz, aromatic), 6.96 (t, 1H, J = 7.6 Hz, aromatic), 7.1–7.2 (m, 3H, aromatic), 7.22 (d, 2H, J = 8.4 Hz, aromatic), 7.3–7.4 (m, 1H, aromatic), 8.09 (d, 1H, J = 8.4 Hz, Apns-NH), 8.3 (br, 1H, benzylamine-NH), 9.39 (s, 1H, benzoyl-OH). Anal. calcd for $C_{34}H_{41}N_3O_6S$: C, 65.89; H, 6.67; N, 6.78; Found C, 65.62; H, 7.00; N, 6.74.

6.1.10. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(4-*n*-propoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10d). Mp 203–205 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 0.98 (t, 3H, J = 7.3 Hz), 1.2–1.3 (br, 2H), 1.33 (s, 3H), 1.52 (s, 3H), 1.73 (q, 2H, J = 6.9 Hz), 2.17 (s, 3H), 2.1–2.3 (m, 1H), 2.6–2.7 (m, 1H), 3.01 (d, 1H, J = 13.2 Hz), 3.91 (t, 2H, J = 6.5 Hz), 4.02–4.08 (m, 1H), 4.1–4.3 (m, 2H), 4.35 (s, 1H), 4.90 (s, 2H), 5.29 (d, 1H, J = 7.8 Hz), 6.84 (d, 3H, J = 8.6 Hz), 6.94–7.07 (m, 4H), 7.11–7.16 (m, 2H), 8.55 (t, 1H, J = 7.8 Hz). Anal. calcd for $C_{27}H_{37}N_3O_4S$: C, 64.90; H, 7.46; N, 8.41; Found C, 64.67; H, 7.64; N, 8.47.

6.1.11. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(4-*n*-propoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12d). Mp 132–135 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 0.96 (t, 3H, J = 7.3 Hz, OnPr-CH_3), 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.66–1.73 (m, 2H, OCH_2–CH_2–CH_3), 1.86 (s, 3H, benzoyl-CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.6–2.8 (m, 2H, Apns-4-CH_2), 3.87 (t, 2H, J = 6.3 Hz, Apns-OCH_2), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.5 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.00 (d, 1H, J = 8.9 Hz, Dmt-2-CH_2), 5.13 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.44 (d, 1H, J = 7.0 Hz, Apns-2-OH), 6.58 (d, 1H, J = 7.6 Hz, aromatic), 6.7–6.8 (m, 3H, aromatic), 6.96 (t, 1H, J = 7.8 Hz, aromatic), 7.1–7.2 (br, 3H, aromatic), 7.26 (d, 2H, J = 8.9 Hz, aromatic), 7.25–7.35 (m, 1H, aromatic), 8.09 (d, 1H, J = 8.4 Hz, Apns-NH), 8.31 (br, 1H, benzylamine-NH), 9.39 (s, 1H, benzoyl-OH). Anal. calcd for $C_{35}H_{43}N_3O_6S$ 0.5 EtOAc: C, 65.56; H, 6.99; N, 6.20; Found C, 65.89; H, 7.05; N, 6.49.

6.1.12. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(4-isopropoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10e). Mp 207–209 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.1–1.2 (br, 2H), 1.26 (d, 6H, J = 5.9 Hz), 1.33 (s, 3H), 1.52 (s, 3H), 2.17 (s, 3H), 2.2–2.3 (m, 1H), 2.6–2.7 (m, 1H), 3.00 (d, 1H, J = 13.2 Hz), 4.0–4.1 (m, 1H), 4.1–4.3 (m, 2H), 4.35 (s, 1H), 4.5–4.6 (m, 1H), 4.90 (s, 2H), 5.28 (d, 1H, J = 7.8 Hz), 6.83 (d, 2H, J = 8.6 Hz), 6.9–7.1 (m, 5H), 7.1–7.2 (m, 1H), 8.53 (t, 1H, J = 5.0 Hz). Anal. calcd for $C_{27}H_{37}N_3O_4S$: C, 64.90; H, 7.46; N, 8.41; Found C, 64.70; H, 7.58; N, 8.30.

6.1.13. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(4-*i*-propoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12e). Mp 133–137 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.22 (s, 3H, *i*Pr-CH_3), 1.24 (s, 3H, *i*Pr-CH_3), 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.84 (s, 3H, benzoyl-CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.6–2.8 (m, 2H, Apns-4-CH_2), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.6 (m, 5H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, Apns-OCH, and Apns-3-CH), 5.01 (d, 1H, J = 8.9 Hz, Dmt-2-CH_2), 5.14 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.44 (d, 1H, J = 6.8 Hz, Apns-2-OH), 6.57 (d, 1H, J = 7.6 Hz, aromatic), 6.75–6.79 (m, 3H, aromatic), 6.95 (t, 1H, J = 7.8 Hz), 7.10–7.14 (m, 3H, aromatic), 7.20 (d, 2H, J = 8.1 Hz, aromatic), 7.28–7.31 (m, 1H, aromatic), 8.09 (d, 1H, J = 8.4 Hz, Apns-NH), 8.31 (bt, 1H, benzylamine-NH), 9.34 (s, 1H, benzoyl-OH). Anal. calcd for $C_{35}H_{43}N_3O_6S$ 0.5 EtOAc: C, 65.56; H, 6.99; N, 6.20; Found C, 65.41; H, 6.90; N, 6.47.

6.1.14. (*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 (10f). 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.

6.1.15. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(3,4-methylenedioxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12f). Mp 133–137 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.87 (s, 3H, benzoyl-CH_3), 2.27 (s, 3H, benzylamine-CH_3), 2.6–2.8 (m, 2H, Apns-4-CH_2), 4.12 (dd, 1H, J = 4.3 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.6 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.00 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.15 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.37 (d, 1H, J = 6.5 Hz, Apns-2-OH), 5.93 (s, 2H, Apns-*O*-C H_2–), 6.59 (d, 1H, J = 7.6 Hz, aromatic), 6.7–6.9 (m, 3H, aromatic), 6.9–7.0 (m, 2H, aromatic), 7.1–7.2 (m, 3H, aromatic), 7.3–7.4 (m, 1H, aromatic), 8.14 (d, 1H, J = 8.9 Hz, Apns-NH), 8.35 (t, 1H, J = 5.4 Hz), 9.40 (s, 1H, benzoyl-OH). Anal. calcd for $C_{33}H_{37}N_3O_7S$ 0.5 EtOAc: C, 63.33; H, 6.23; N, 6.33; Found C, 63.06; H, 5.99; N, 6.68.

6.1.16. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(4-methylphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10g). Mp 207–209 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.1–1.3 (br, 2H), 1.33 (s, 3H), 1.52 (s, 3H), 2.16 (s, 3H), 2.2–2.3 (m, 1H), 2.29 (s, 3H), 2.6–2.7 (br, 1H), 3.04 (d, 1H, J = 12.7 Hz), 4.0–4.1 (m, 1H), 4.1–4.3 (m, 2H), 4.35 (s, 1H), 4.90 (s, 2H), 5.29–5.32 (br, 1H), 6.93–7.12 (m, 8H), 8.54 (t, 1H, J = 5.1 Hz). Anal. calcd for $C_{25}H_{33}N_3O_3S$: C, 65.90; H, 7.30; N, 9.22; Found C, 65.77; H, 7.48; N, 9.41.

6.1.17. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(4-methylphenyl)-butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12g). Mp 142–146 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.86 (s, 3H, benzoyl-CH_3), 2.25 (s, 3H, Apns-CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.6–2.9 (m, 2H, Apns-4-CH_2), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.5 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.00 (d, 1H, J = 9.5 Hz, Dmt-2-CH_2), 5.12 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.47 (d, 1H, J = 6.2 Hz, Apns-2-OH), 6.57 (d, 1H, J = 7.3 Hz, aromatic), 6.78 (d, 1H, J = 7.8 Hz, aromatic), 6.96 (t, 1H, J = 7.8 Hz), 7.0–7.2 (m, 5H, aromatic), 7.20 (d, 2H, J = 7.6 Hz, aromatic), 7.28–7.31 (m, 1H, aromatic), 8.08 (d, 1H, J = 7.8 Hz, Apns-NH), 8.30 (br, 1H, benzylamine-NH), 9.39 (s, 1H, benzoyl-OH). Anal. calcd for $C_{33}H_{39}N_3O_5S$ 0.5 EtOAc: C, 66.33; H, 6.84; N, 6.63; Found C, 66.67; H, 6.93; N, 6.96.

6.1.18. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(3-methylphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10h). Mp 173–175 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.1–1.3 (br, 2H), 1.33 (s, 3H), 1.52 (s, 3H), 2.16 (s, 3H), 2.2–2.3 (m, 1H), 2.29 (s, 3H), 2.6–2.7 (br, 1H), 3.06 (d, 1H, J = 12.9 Hz), 4.0–4.1 (m, 1H), 4.1–4.3 (m, 2H), 4.36 (s, 1H), 4.91 (s, 2H), 5.3–5.4 (br, 1H), 6.91–7.04 (m, 5H), 7.13–7.20 (m, 3H), 8.54 (t, 1H, J = 5.1 Hz). Anal. calcd for $C_{25}H_{33}N_3O_3S$: C, 65.90; H, 7.30; N, 9.22; Found C, 65.67; H, 7.42; N, 9.39.

6.1.19. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(3-methyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12h). Mp 179–181 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.87 (s, 3H, benzoyl- CH_3), 2.24 (s, 3H, Apns-CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.65–2.85 (m, 2H, Apns-4-CH_2), 4.11 (dd, 1H, J = 4.3 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.5 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.01 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.13 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.49 (d, 1H, J = 6.8 Hz, Apns-2-OH), 6.57 (d, 1H, J = 6.8 Hz, aromatic), 6.79 (d, 1H, J = 7.3 Hz, aromatic), 6.96 (t, 2H, J = 7.6 Hz, aromatic), 7.0–7.2 (m, 6H, aromatic), 7.29–7.30 (m, 1H, aromatic), 8.13 (d, 1H, J = 8.4 Hz, Apns-NH), 8.32 (bt, 1H, benzylamine-NH), 9.40 (s, 1H, benzoyl-OH). Anal. calcd for $C_{33}H_{39}N_3O_5S$ 0.5 EtOAc: C, 66.33; H, 6.84; N, 6.63; Found C, 66.52; H, 6.73; N, 6.82.

6.1.20. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(4-chlorophenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10i). Mp 201–203 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.2–1.4 (br, 2H), 1.33 (s, 3H), 1.52 (s, 3H), 2.17 (s, 3H), 2.28 (t, 1H, J = 11.6 Hz), 2.65 (t, 1H, J = 8.2 Hz), 3.0–3.1 (br, 1H), 4.0–4.1 (m, 1H), 4.1–4.3 (m, 2H), 4.36 (s, 1H), 4.90 (s, 2H), 5.33 (d, 1H, J = 7.8 Hz), 6.98 (s, 2H), 7.16 (d, 2H, J = 5.9 Hz), 7.1–7.3 (br, 1H), 7.35 (d, 2H, J = 8.1 Hz), 8.51 (t, 1H, J = 5.1 Hz). Anal. calcd for $C_{24}H_{30}ClN_3O_3S$: C, 60.55; H, 6.35; N, 8.83; Found C, 60.20; H, 6.46; N, 8.75.

6.1.21. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(4-chlorophenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12i). Mp 143–146 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.82 (s, 3H, benzoyl- CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.7–2.9 (m, 2H, Apns-4-CH_2), 4.14 (dd, 1H, J = 4.3 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.5 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.00 (d, 1H, J = 9.5 Hz, Dmt-2-CH_2), 5.15 (d, 1H, J = 9.2 Hz, Dmt-2-CH_2), 5.47 (d, 1H, J = 6.5 Hz, Apns-2-OH), 6.57 (d, 1H, J = 7.3 Hz, aromatic), 6.78 (d, 1H, J = 7.8 Hz, aromatic), 6.96 (t, 1H, J = 7.8 Hz, aromatic), 7.0–7.2 (m, 3H, aromatic), 7.2–7.4 (m, 5H, aromatic), 8.17 (d, 1H, J = 8.6 Hz, Apns-NH), 8.34 (bt, 1H, benzylamine-NH), 9.40 (s, 1H, benzoyl-OH). Anal. calcd for $C_{32}H_{36}ClN_3O_5S$: C, 62.99; H, 5.95; N, 6.89; Found C, 62.74; H, 6.17; N, 6.65.

6.1.22. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(3-chlorophenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10j). Mp 161–163 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.2–1.4 (br, 2H), 1.34 (s, 3H), 1.52 (s, 3H), 2.17 (s, 3H), 2.23–2.32 (m, 1H), 2.66 (t, 1H, J = 8.2 Hz), 3.06 (d, 1H, J = 11.1 Hz), 4.0–4.1 (m, 1H), 4.1–4.3 (m, 2H), 4.36 (s, 1H), 4.90 (s, 2H), 5.32 (d, 1H, J = 8.1 Hz), 6.96 (s, 2H), 7.09–7.36 (m, 6H), 8.48 (t, 1H, J = 5.1 Hz). Anal. calcd for $C_{24}H_{30}ClN_3O_3S$: C, 60.55; H, 6.35; N, 8.83;; Found C, 59.72; H, 6.48; N, 8.72.

6.1.23. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(3-chlorophenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide

(12j). Mp 201–202 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.35 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.83 (s, 3H, benzoyl-CH_3), 2.27 (s, 3H, benzylamine-CH_3), 2.67–2.85 (m, 2H, Apns-4-CH_2), 4.16 (dd, 1H, J = 4.6 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.6 (m, 4H, benzylamine-CH_2, Dmt-4-CH, Apns-2-CH, and Apns-3-CH), 5.00 (d, 1H, J = 8.9 Hz, Dmt-2-CH_2), 5.17 (d, 1H, J = 8.9 Hz, Dmt-2-CH_2), 5.44 (d, 1H, J = 6.8 Hz, Apns-2-OH), 6.58 (d, 1H, J = 7.0 Hz, aromatic), 6.79 (d, 1H, J = 7.6 Hz, aromatic), 6.97 (t, 2H, J = 7.7 Hz, aromatic), 7.0–7.2 (m, 3H, aromatic), 7.20–7.33 (m, 4H, aromatic), 7.40 (s, 1H, aromatic), 8.24 (d, 1H, J = 8.4 Hz, Apns-NH), 8.38 (bt, 1H, benzylamine-NH), 9.41 (s, 1H, benzoyl-OH). Anal. calcd for $C_{32}H_{36}ClN_3O_5S$: C, 62.99; H, 5.95; N, 6.89; Found C, 62.86; H, 5.99; N, 6.80.

6.1.24. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(4-fluorophenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10k). Mp 197–199 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.1–1.3 (br, 2H), 1.33 (s, 3H), 1.52 (s, 3H), 2.17 (s, 3H), 2.2–2.3 (m, 1H), 2.6–2.7 (m, 1H), 3.05 (d, 1H, J = 11.1 Hz), 4.0–4.1 (m, 1H), 4.1–4.3 (m, 2H), 4.36 (s, 1H), 4.90 (s, 2H), 5.31 (d, 1H, J = 8.1 Hz), 6.95–6.99 (m, 3H), 7.07–7.20 (m, 5H), 8.52 (t, 1H, J = 5.0 Hz). Anal. calcd for $C_{24}H_{30}FN_3O_3S$: C, 62.72; H, 6.58; N, 9.14; Found C, 62.47; H, 6.80; N, 9.18.

6.1.25. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(4-fluorophenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12k). Mp 190–195 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.36 (s, 3H, Dmt-5-CH_3), 1.51 (s, 3H, Dmt-5-CH_3), 1.82 (s, 3H, benzoyl-CH_3), 2.27 (s, 3H, benzylamine-CH_3), 2.68–2.86 (m, 2H, Apns-4-CH_2), 4.14 (dd, 1H, J = 4.9 Hz, 14.9 Hz, benzylamine-CH_2), 4.3–4.5 (m, 4H, Apns-3-CH, benzylamine-CH_2, Dmt-4-CH, and Apns-2-CH), 5.01 (d, 1H, J = 8.9 Hz, Dmt-2-CH_2), 5.17 (d, 1H, J = 8.9 Hz, Dmt-2-CH_2), 5.43 (d, 1H, J = 7.0 Hz, Apns-2-OH), 6.56 (d, 1H, J = 7.3 Hz, aromatic), 6.79 (d, 1H, J = 8.1 Hz, aromatic), 6.94–7.01 (m, 2H, aromatic), 7.09–7.18 (m, 5H, aromatic), 7.23–7.32 (m, 3H, aromatic), 8.23 (d, 1H, J = 8.4 Hz, Apns-NH), 8.38 (t, 1H, J = 5.5 Hz, benzylamine-NH), 9.40 (s, 1H, benzoyl-OH). Anal. calcd for $C_{32}H_{36}FN_3O_5S$: C, 64.74; H, 6.11; N, 7.08; Found C, 64.58; H, 6.12;N, 7.21.

6.1.26. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-(3-fluorophenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10l). Mp 187–189 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.2–1.3 (br, 2H), 1.34 (s, 3H), 1.52 (s, 3H), 2.17 (s, 3H), 2.2–2.3 (m, 1H), 2.6–2.7 (m, 1H), 3.07 (d, 1H, J = 10.5 Hz), 4.0–4.1 (m, 1H), 4.1–4.3 (m, 2H), 4.37 (s, 1H), 4.90 (s, 2H), 5.32 (d, 1H, J = 8.4 Hz), 6.94–7.06 (m, 6H), 7.1–7.2 (m, 1H), 7.29–7.37 (m, 1H), 8.50 (t, 1H, J = 5.1 Hz). Anal. calcd for $C_{24}H_{30}FN_3O_3S$: C, 62.72; H, 6.58; N, 9.14;; Found C, 62.47; H, 6.67; N, 9.04.

6.1.27. (*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(3-fluorophenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12l). Mp 171–173 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.36 (s, 3H, Dmt-5-CH_3), 1.50 (s, 3H, Dmt-5-CH_3), 1.82 (s, 3H, benzoyl-CH_3), 2.26 (s, 3H, benzylamine-CH_3), 2.66–2.84 (m, 2H, Apns-4-CH_2), 4.12 (dd, 1H, J = 4.3 Hz, 15.1 Hz, benzylamine-CH_2), 4.3–4.6 (m, 4H, Apns-3-CH, benzylamine-CH_2, Dmt-4-CH, and Apns-2-CH), 5.01 (d, 1H, J = 8.9 Hz, Dmt-2-CH_2), 5.16 (d, 1H, J = 8.9 Hz, Dmt-2-CH_2), 5.44 (d, 1H, J = 6.8 Hz, Apns-2-OH), 6.56 (d, 1H, J = 7.0 Hz, aromatic), 6.78 (d, 1H, J = 7.6 Hz, aromatic), 6.96 (t, 1H, J = 7.7 Hz, aromatic), 7.0–7.2 (m, 6H, aromatic), 7.2–7.4 (m, 3H, aromatic), 8.18 (d, 1H, J = 8.4 Hz, Apns-NH), 8.35 (t, 1H, J = 5.4 Hz, benzylamine-NH), 9.40 (s, 1H, benzoyl-OH). Anal. calcd for $C_{32}H_{36}FN_3O_5S$ 0.5 EtOAc: C, 64.03; H, 6.32; N, 6.59; Found C, 64.30; H, 6.13; N, 6.99.

6.1.28. (*R*)-*N*-(2,6-Dimethylbenzyl)-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (9b). To a solution of (*R*)-*N*-*tert*-butoxycarbonyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (15.7 g, 60 mmol) and triethylamine (8.76 mL, 63 mmol) in ethyl acetate (230 mL), diphenylphosphoryl chloridate (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-methylbenzylamine hydrochloride (12.1 g, 63 mmol), and triethylamine (18.3 mL, 132 mmol) were added in an ice-bath, followed by overnight stirring. The reaction mixture was then 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), to which was added 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.

6.1.29. (*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 (10m). To a solution of compound **9b** (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), and 1-hydroxybenztriazole (68 mg, 0.50 mmol) in ethyl acetate (5 mL), *N*,*N*′-dicyclohexylcarbodiimide (113 mg, 0.55 mmol) was added, followed by stirring overnight. The reaction mixture was filtered, and the filtrate was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine. Then it was dried over $MgSO_4$, filtered, and concentrated. The residue was redissolved in ethyl acetate (5 mL), and subsequently stirred for 1 h with 4 N HCl in ethyl acetate (5 mL). The reaction mixture was 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 a precipitate. The crude product was recrystallized from ethyl acetate, giving 129 mg of the title compound, in 53% yield: 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; Found C, 62.00; H, 7.40; N, 8.34.

6.1.30. (*R*) - *N* - (2,6 - Dimethylbenzyl) - 3 - [(2*S*,3*S*) - 2 - hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(4-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12m). Mp 218–220 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.35 (s, 3H, Dmt-5-CH_3), 1.45 (s, 3H, Dmt-5-CH_3), 1.84 (s, 3H, benzoyl-CH_3), 2.31 (s, 6H, benzylamine-CH_3), 2.6–2.8 (m, 2H, Apns-4-CH_2), 3.72 (s, 3H, Apns-OCH_3), 4.17 (dd, 1H, $J = 2.7$ Hz, 13.2 Hz, benzylamine-CH_2), 4.2–4.4 (m, 1H, Apns-3-CH), 4.43–4.54 (m, 3H, benzylamine-CH_2, Dmt-4-CH, and Apns-2-CH), 4.98 (d, 1H, $J = 8.9$ Hz, Dmt-2-CH_2), 5.17 (d, 1H, $J = 9.2$ Hz, Dmt-2-CH_2), 5.28 (d, 1H, $J = 7.0$ Hz, Apns-2-OH), 6.58 (d, 1H, $J = 6.8$ Hz, aromatic), 6.81 (t, 3H, $J = 9.3$ Hz, aromatic), 6.93–7.13 (m, 4H, aromatic), 7.30 (d, 1H, $J = 8.6$ Hz, aromatic), 8.05–8.11 (br, 1H, benzylamine-NH), 8.21 (d, 1H, $J = 8.4$ Hz, Apns-NH), 9.39 (s, 1H, benzoyl-OH). Anal. calcd for $C_{34}H_{41}N_3O_6S$: C, 65.89; H, 6.67; N, 6.78; Found C, 65.49; H, 6.74; N, 6.75.

6.1.31. (*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 (10n). 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.

6.1.32. (*R*) - *N* - (2,6 - Dimethylbenzyl) - 3 - [(2*S*,3*S*) - 2 - hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(3-methoxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12n). Mp 200–202 °C, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.35 (s, 3H, Dmt-5-CH_3), 1.46 (s, 3H, Dmt-5-CH_3), 1.85 (s, 3H, benzoyl-CH_3), 2.31 (s, 6H, benzylamine-CH_3), 2.6–2.8 (m, 2H, Apns-4-CH_2), 3.75 (s, 3H, Apns-OCH_3), 4.18 (dd, 1H, $J = 3.5$ Hz, 14.0 Hz, benzylamine-CH_2), 4.2–4.4 (m, 1H, Apns-3-CH), 4.42–4.55 (m, 3H, benzylamine-CH_2, Dmt-4-CH, and Apns-2-CH), 4.99 (d, 1H, $J = 8.6$ Hz, Dmt-2-CH_2), 5.18 (d, 1H, $J = 8.6$ Hz, Dmt-2-CH_2), 5.27 (d, 1H, $J = 7.0$ Hz, Apns-2-OH), 6.59 (d, 1H, $J = 6.8$ Hz, aromatic), 6.72–6.80 (m, 2H, aromatic), 6.93–7.11 (m, 6H, aromatic), 7.16 (t, 1H, $J = 7.8$ Hz, aromatic), 8.08–8.12 (br, 1H, benzylamine-NH), 8.25 (d, 1H, $J = 8.4$ Hz, Apns-NH), 9.39 (s, 1H, benzoyl-OH). Anal. calcd for $C_{34}H_{41}N_3O_6S\ H_2O$: C, 64.03; H, 6.80; N, 6.59; Found C, 64.19; H, 6.50; N, 6.38.

6.1.33. (*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 (10o). 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.

6.1.34. (*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzoyl)amino-4-(3,4-methylenedioxyphenyl)butanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12o). Mp 201–203 °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), 1.86 (s, 3H, benzoyl-CH_3), 2.31 (s, 6H, benzylamine-CH_3), 2.56–2.73 (m, 2H, Apns-4-CH_2), 4.18 (dd, 1H, $J = 2.7$ Hz, 13.8 Hz, benzylamine-CH_2), 4.2–4.3 (m, 1H, Apns-2-CH), 4.45–4.54 (m, 3H, benzylamine-CH_2, Dmt-4-CH, and Apns-3-CH), 4.98 (d, 1H, $J = 9.2$ Hz, Dmt-2-CH_2), 5.17–5.24 (m, 2H, Dmt-2-CH_2, Apns-2-OH), 5.94 (s, 2H, O-CH_2-O), 6.59 (d, 1H, $J = 6.8$ Hz, aromatic), 6.81 (t, 3H, $J = 8.1$ Hz, aromatic), 6.86–7.11 (m, 6H, aromatic), 8.09–8.13 (br, 1H, benzylamine-NH), 8.23 (d, 1H, $J = 8.6$ Hz, Apns-NH), 9.41 (s, 1H, benzoyl-OH). Anal. calcd for $C_{34}H_{39}N_3O_7S$ 0.5 EtOAc: C, 63.79; H, 6.39; N, 6.20; Found C, 63.70; H, 6.23; N, 6.53.

6.2. In vitro metabolism experiment

Metabolic stability was determined by using liver microsomes isolated from humans, rats or dogs. The reaction mixture contained 0.1 M phosphate buffer (pH 7.4), 5 μM test compound, 5 mM $MgCl_2$, 1 mM NADPH and/or 5 mM UDPGA, and 0.5 mg/mL microsomes in the presence or absence of ritonavir (0.5 or 2.5 μM) in a total volume of 0.2 mL. The incubation was initiated by the addition of microsomes and was performed for 30 min at 37 °C. The reaction was terminated by adding 0.4 mL of ice-cold methanol, and the reaction mixture was then centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was filtered through a PTFE filter, and the residual amount of the test compound in the supernatant was determined by LC/UV analysis. The mean percentage (%) of the test compound was remaining calculated from the data of duplicate experiments.

6.3. Pharmacokinetics

Pharmacokinetics parameters of the drugs were studied in dogs. The pharmacokinetic behavior of **12n** was evaluated following a single oral and intravenous dose to three male beagle dogs (7–10 kg, HRP, Inc., Cumberland, USA). Compound **12n** dissolved in 50% PEG was administered intravenously via the cepatic vein as a bolus injection at the dose of 25 mg/kg, and the same dose was administered orally by gavage. Heparinized blood samples were collected from a bolus via the cepatic vein at designated times after the dosing. Two hundred microliters of plasma was obtained by immediate centrifugation and kept frozen at −80 °C until analyzed. The plasma sample was vortexed with 4 mL of *tert*-buthylmethylether, and centrifuged at

3500 rpm for 20 min. A portion of the organic layer obtained (3.6 mL) was evaporated to dryness at 40 °C, and then reconstituted in 0.3 mL of 50% methanol. Two hundred microliters of the reconstituted sample was injected into an HPLC system equipped with a Zorbax bonus RP column (4.6 × 150 mm), and the drug was eluted with a linear gradient from 50 to 74% of acetonitrile in 0.1% trifluoroacetic acid (TFA) at a flow rate of 1.0 mL/min and monitored with UV detection at 210 nm. The drug concentration was calculated from the peak area using the calibration curve obtained from determination of standard plasma samples (The lower quantification limit was 10 ng/mL, and a maximum for the range was 10,000 ng/mL). Pharmacokinetic parameters for inhibitors were estimated by using a non-compartmental method. Maximum plasma concentration (C_{max}), and time of maximum plasma concentration (T_{max}) were determined by inspection of individual subject concentration–time curves, and the mean area under the plasma concentration–time curve (AUC) was determined by the linear trapezoidal rule. The apparent plasma half-life $\left(t_{1/2\beta}\right)$ was estimated from the slope of the terminal phase fitted to the log plasma concentration–time data by the method of least squares. The apparent distribution volume (V_{dss}) of the inhibitor was determined by the following equation.

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

where $AUMC$ (0–∞) is the total area under the first moment of the drug concentration curve from zero to infinity. The plasma clearance (CL) was calculated as the dose divided by the AUC from zero to infinity $[AUC]_0^\infty$.

6.4. HIV protease inhibition

Recombinant wild-type HIV protease (NY5-type sequence) was expressed in *Escherichia coli* and purified by column chromatography to a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Synthetic peptide substrate [H-Lys-Ala-Arg-Val-Tyr-Phe(4-NO_2)-Glu-Ala-Nle-NH_2] was purchased from Bachem Inc., Switzerland. In the inhibition assay, different concentrations of test compounds dissolved in dimethylsulfoxide were pre-incubated at 37 °C for 5 min with HIV-1 proteases in 200 mM 2-[*N*-morpholino]ethanesulfonic acid (MES-NaOH) buffer, pH 5.5, containing 1 M NaCl, 2 mM dithiothreitol and 2 mM EDTA-2Na. Then the enzymic reactions were initiated by the addition of 0.1 mM synthetic peptide substrate, and incubated at 37 °C for 15 min. The reactions were stopped by the addition of 1 N HCl. The C-terminal cleavage fragment was separated by reverse-phase HPLC on a C_{18} column operated under isocratic conditions (2 mL/min, 10% acetonitrile aq containing 0.1% TFA), detected by fluorometry (excitation at 275 nm; emission at 305 nm), and quantified by using a synthetic product standard. The inhibition constant, K_i, was analyzed by a mathematical model for tight-binding inhibitors.[19] Briefly, the initial velocity data on HIV protease in the presence of different concentrations of test compounds were fitted by nonlinear regression analysis to Eq. 1 with KaleidaGraph software for Macintosh, where V is the initial velocity with different concentrations of test compounds; V_0 is the initial velocity in the absence of the inhibitor; K_m is the Michaelis constant; and S, Et, and It are the concentrations of substrate, active enzyme, and inhibitor, respectively:

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

6.5. Antiviral activity

Evaluation of the in vitro antiviral activities of the test compounds against a wild-type HIV-1 (strain IIIB) and drug-resistant HIV containing multi-mutations isolated from patients were conducted as described previously.[18] The virus-infected CEM-SS cells were incubated with different concentrations of test compounds in the absence or presence of 50% human serum at 37 °C for 6 days in a 5% CO_2 incubator. Then virus-induced cytopathic effects were analyzed by staining with the tetrazolium dye XTT. The concentration of test compounds affording 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}). In the case of the experiments with drug-resistant HIV, fresh human peripheral blood lymphocytes (PBMC) were used for the target cells. The virus infected PBMC were incubated at 37 °C for 7 days in a 5% CO_2 incubator with different concentrations of test compounds in RPMI 1640 medium containing 15% fetal bovine serum. Then transcriptase activity derived from the virus in the cell-free supernatant was measured by polymerase chain reaction assay. The concentration of test compounds achieving 50% inhibition of the transcriptase activity in the cell-free supernatant was defined as the EC_{50}.

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