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Locking the two ends of tetrapeptidic HTLV-I protease inhibitors inside the enzyme

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ABSTRACT

Adult T-cell leukemia and tropical spastic paraparesis/HTLV-I-associated myelopathy are only some of the more common end results of an infection with a human T-cell leukemia virus type 1 (HTLV-I). Expanding from our previous reports, we synthesized all different permutations of tetrapeptidic HTLV-I protease inhibitors using at least eight P_3-cap and five P'_1-cap moieties. The inhibitors exhibited over 97% inhibition against HIV-1 protease and a wide range of inhibitory activity against HTLV-I protease.

1. Introduction

Identified in the laboratories of R.C. Gallo in the 1980s from patients with adult T-cell leukemia (ATL), the human T-cell leukemia virus type 1 (HTLV-I) was the first human retrovirus to be clearly linked with ATL, HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), HTLV-I uveitis (HAU), HTLV-I-associated arthropathy (HAAP) and a number of other chronic diseases.[1–3] HTLV-I infection is currently considered intractable, because 20–30 million individuals worldwide are viral carriers, of whom roughly 2–6% will succumb to the disease after a long latent period of 20–50 years.[4,5] HTLV-I infection is endemic in southwestern Japan, Central Africa, Caribbean Islands, South America and other isolated populations in the world.[6] In southwestern Japan, where the virus is most prevalent in the world, antibodies to HTLV-I are found in 6–37% of healthy adults.[7] Estimations showed that 1.2 million Japanese people are infected with HTLV-I, and more than 700 cases of ATL are being diagnosed each year in Japan. HTLV-I is not transmitted by casual contact because the three main routes of viral transmission require close contact with infected T-lymphocytes.[8–10] These routes include vertical transmission from mother to child through breast-feeding; horizontal transmission by sexual intercourse; and exposure to contaminated blood, either through blood transfusion or sharing of contaminated needles. Despite significant advances in understanding the pathogenesis of HTLV-I-associated diseases and the wide experience with the virus over the past twenty years, ATL patients generally have a very poor prognosis and the disease remains difficult to treat.

HTLV-I is classified as a member of the family *Retroviridae*, which also includes the human immunodeficiency virus (HIV).[11] The virally encoded homodimeric aspartic protease, which is composed of two identical chain-subunits with each chain containing 125 amino acid residues which is roughly 25% larger than the HIV protease, mediates crucial proteolytic processing of viral protein precursors at the late stage of viral replication of the virus and thus represents a virus-specific target for HTLV-I infection.[12,13] The three main major proteins, Gag, Pol and Env, are encoded within the retroviral genome.[12,14] Upon viral maturation, the retroviral protease cleaves the Gag protein into matrix (MA), capsid (CA) and nucleocapsid (NC) proteins, while proteolytic cleavage of the Pol precursor yields reverse transcriptase/ribonuclease H (RT–RH) and integrase (IN). The third polyprotein, Env, contains two envelop proteins, surface glycoprotein (SU) and transmembrane protein (TM), that are associated with the host cell-derived virus membrane. Although HTLV-I protease shares 45% sequence identity with HIV-1 protease at the substrate binding region and various HIV-1 protease inhibitors are presently being used successfully in therapies against AIDS, analogous HIV-1 protease inhibitors in the treatment of HTLV-I infections do not cure ATL and HTLV-I

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infections because of large differences in the specificity of the two enzymes.[15–17] HTLV-I protease continues to be one of the primary targets of ATL drug discovery due to its central role in processing of viral polypeptide precursors. The role of HTLV-I protease inhibitors is just beginning to be investigated.

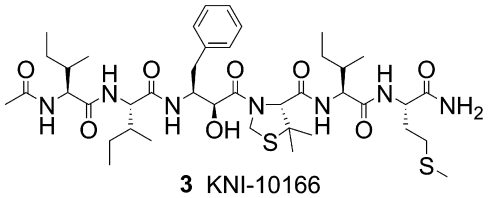
Our research centres around HTLV-I protease inhibitors that contain an allophenylnorstatine [(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid, Apns] residue as a hydroxymethylcarbonyl isostere derived from a natural scissile amino acid sequence 'Leu-Pro' (**1**),[18] which is the HTLV-I protease MA and CA cleavage site region based on the concept of substrate-transition state mimicry, and from which we derived potent peptidic HTLV-I protease inhibitors KNI-10161 (**2**, IC_{50} = 159 nM),[19] as shown in Figure 1. Believing that smaller peptidic inhibitors with few hydrophilic moieties would more readily penetrate intact cells, we began our natural amino acid optimization studies and arrived at inhibitor KNI-10166 (**3**, IC_{50} = 88 nM).[20] Considering that the efficacy of peptides as in vivo agents is severely compromising by their susceptibility to proteolytic degradation and difficulty in penetrating intact cells, we explored non-natural amino acid residues and further reduce the size of the inhibitor in inhibitors KNI-10247 (**4**, IC_{50} = 144 nM).[21] After further optimization of compound **4** for inhibitory activity against HTLV-I protease, we reported a series of potent HTLV-I protease inhibitors that possessed an isobutylamine P'_1-cap moiety and various hydrophobic substituents at the P_3-cap position.[22] Although almost all of the inhibitors were highly potent with IC_{50} values ranging from 79 to 107 nM, the results from the structure–activity relationship study remained ambiguous due to the narrow range of inhibitory activity against HTLV-I protease. In the work described herein, using at least eight P_3-cap and five P'_1-cap hydrophobic moieties, we applied combinatorics to the synthesis of all possible permutations to compounds that possessed a similar $P_3 - P'_1$ sequence as inhibitor **4**. As a result of varying both the P_3-cap and P'_1-cap moieties in our attempts to 'lock' the two ends and trap the inhibitor within the enzyme, we developed potent tetrapeptidic HTLV-I protease inhibitors and obtained a statistically reliable quantitative structure–activity relationship (QSAR) equation to support the observed structure–activity trends.

2. Chemistry

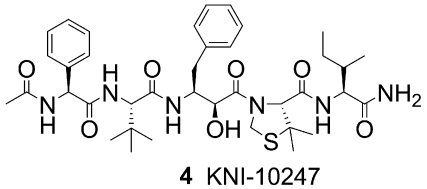
Compounds **8aa–8id** were prepared by standard solution phase peptide coupling chemistry (Scheme 1). A respective alkylamine was coupled with N^{α}-*tert*-butyloxycarbonyl (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (Boc-Dmt-OH) using benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) in the presence of triethylamine (TEA), followed by Boc-deprotection with 4 N HCl in dioxane to obtain general compound **5**. General compound **6** was prepared by coupling general compound **5** and Boc-Apns-OH with BOP. The same deprotection/condensation procedure was repeated for the successive introduction of N^{α}-*tert*-butyloxycarbonyl L-*tert*-leucine (Boc-Tle-OH) and N^{α}-*tert*-butyloxycarbonyl L-(+)-α-phenylglycine (Boc-Phg-OH) to afford compounds **8aa–8af**, that were coupled one more time with a respective carboxylic acid to afford compounds **8ba–8id**.

1 substrate H-Pro-Gln-Val- Leu -Pro-Val-Met-His-Pro-OH

2 KNI-10161 H-Pro-Gln-Val-Apns-Pro-Val-Met-His-OH



3 KNI-10166



4 KNI-10247

Figure 1. From an HTLV-I protease substrate (**1**), potent HTLV-I protease inhibitors, KNI-10161 (**2**), KNI-10166 (**3**) and KNI-10247 (**4**), were developed.

3. Results and discussion

HTLV-I protease inhibitory activity assay was performed using HTLV-I protease L40I mutant as previously reported.[21,22] The L40I mutation was necessary to prevent autolysis, thereby improving the stability of the enzyme.[23] In a preliminary report, we described the HTLV-I protease inhibition of tetrapeptidic inhibitors having the general structures of Ac-Phg-Tle-Apns-Dmt-R where the α-carbon of the P'_1-cap moiety was unbranched (**8ba**,**bb**,**bc**) (Table 1).[21] In the current work, we studied the effect of branching at the α-carbon of the P'_1-cap moiety and lengthening the P'_1-cap moiety. Among inhibitors **8ba**, **8bb** and **8bc**, an isobutylamino P'_1-cap moiety seemed to enhanced HTLV-I protease inhibitory activity, and consequently, we reported very potent HTLV-I protease inhibitors possessing an isobutylamino P'_1-cap moiety and various P_3-cap moieties (**8ab**,**bb**,**cb**,**db**,**eb**,**fb**,**gb**,**hb**).[22] However, within the isobutylamino P'_1-cap moiety series, the inhibition range was fairly narrow (IC_{50} = 83–107 and 332 nM), thereby suggesting that our structure–activity relationship observations could be fairly speculative. To clarify our deduced structure–activity relationships trend, in the current study, we varied the P'_1-cap moiety along with the P_3-cap moiety, and statistically validated the observed trends (Eq. 1). Among the 44 inhibitors that are shown in Table 1, 34 compounds have not been previously described.

Quantitative structure–activity relationship equation correlating HTLV-I protease inhibition and structural features with enzyme inhibition, IC_{50} (nM).

$$-\log(IC_{50}) = 0.520(P_3\text{-cap moiety}) + 0.269(P'_1\text{-cap moiety}) - 2.665 \quad (1)$$

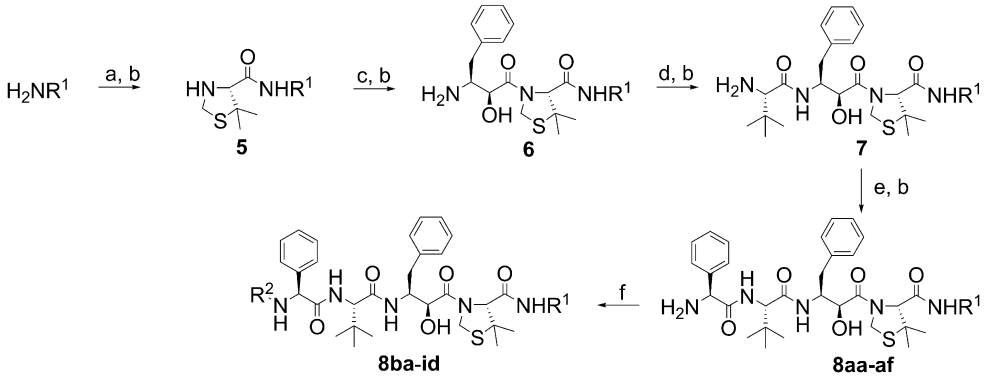
$$n = 42, r^2 = 0.87, F = 131, p = 0.001$$

where P_3-cap moiety : $H = 0.000, Ac = 0.761, EtCO = 0.817,$ $^{n}PrCO = 1.000, ^{c}PrCO = 0.986, ^{i}PrCO = 0.977, ^{t}BuCO = 0.846,$ $^{i}BuCO = 0.975$, ranging from 0 to 1.

P'_1-cap moiety : $CH_2{}^{c}Pr = 0.000, ^{i}Bu = 0.734, ^{t}Pe = 1.000,$ $(R) - CH(Me)^{i}Pr = 0.454, (R) - CH(Me)^{t}Bu = 0.923, ^{i}Pe = 0.484,$ ranging from 0 to 1.

Equation 1. Quantitative structure–activity relationship equation correlating HTLV-I protease inhibition and structural features with enzyme inhibition, IC_{50} (nM).

We derived a QSAR equation (Eq. 1) to correlate chemical structure and HTLV-I protease inhibition. At first glance, it was apparent that compound **8dd** did not fit the HTLV-I inhibition trend due to its very low inhibitory activity, and the compound was consequently excluded as an outlier (Fig. 2). The scores given for the P_3-cap and P'_1-cap moieties were awarded on averaged normalized percentile based on IC_{50} values. In simpler words, groups of compounds, where only one portion of the structure was changed (either the P_3-cap or P'_1-cap moiety), were ranked as normalized percentiles from 0 to 1, and based on their IC_{50} values. To compare between the groups, the average of the respective normalized per-



Scheme 1. Synthesis of compounds **8aa–8id**. Reagents and conditions: (a) Boc-Dmt-OH, BOP, TEA, DMF, rt, 15 h; (b) 4 N HCl/dioxane, rt, 1 h; (c) Boc-Apns-OH, BOP, TEA, DMF, rt, 15 h; (d) Boc-Tle-OH, BOP, TEA, DMF, rt, 15 h; (e) Boc-Phg-OH, BOP, TEA, DMF, rt, 15 h; (f) R^2OH, BOP, TEA, DMF, rt, 15 h.

Table 1
Structures and IC_{50} values (nM) of HTLV-I protease inhibitors **8aa–8id**

R^2	R^1					
	$CH_2{}^cPr$	^{i}Bu	^{t}Pe	(*R*)-CH(Me)^{i}Pr	(*R*)-CH(Me)^{t}Bu	^{i}Pe
H	**8aa**, KNI-10679, 928 (462)	**8ab**, KNI-10673, 332 (293)	**8ac**, KNI-10680, 180 (249)	**8ad**, KNI-10681, 354 (349)	**8ae**, KNI-10683, 156 (261)	**8af**, KNI-10684, 372 (342)
Ac	**8ba**, KNI-10592, 146 (186)	**8bb**, KNI-10516, 107 (118)	**8bc**, KNI-10600, 113 (100)	**8bd**, KNI-10586, 136 (140)	**8be**, KNI-10627, 106 (105)	NA
EtCO	**8ca**, KNI-10589, 145 (174)	**8cb**, KNI-10571, 105 (110)	**8cc**, KNI-10599, 98 (94)	**8cd**, KNI-10577, 129 (131)	**8ce**, KNI-10578, 103 (98)	NA
^{n}PrCO	**8da**, KNI-10591, 113 (140)	**8db**, KNI-10570, 104 (89)	**8dc**, KNI-10635, 74 (75)	**8dd**, KNI-10587, 659 (105)	**8de**, KNI-10588, 94 (79)	**8df**, KNI-10671, 95 (103)
^{c}PrCO	**8ea**, KNI-10670, 142 (142)	**8eb**, KNI-10575, 97 (90)	**8ec**, KNI-10649, 86 (76)	**8ed**, KNI-10601, 90 (107)	**8ee**, KNI-10628, 85 (80)	NA
^{i}PrCO	**8fa**, KNI-10613, 134 (144)	**8fb**, KNI-10574, 94 (91)	**8fc**, KNI-10636, 78 (77)	**8fd**, KNI-10688, 122 (108)	**8fe**, KNI-10590, 84 (81)	NA
^{t}BuCO	**8ga**, KNI-10669, 187 (168)	**8gb**, KNI-10572, 83 (107)	**8gc**, KNI-10631, 97 (90)	**8gd**, KNI-10602, 133 (127)	**8ge**, KNI-10630, 97 (95)	NA
^{i}BuCO	**8ha**, KNI-10668, 132 (144)	**8hb**, KNI-10573, 102 (91)	**8hc**, KNI-10648, 82 (77)	**8hd**, KNI-10667, 107 (109)	**8he**, KNI-10629, 84 (81)	**8hf**, KNI-10672, 107 (107)
$CH_2{=}CH(CH_2)_2CO$	NA	NA	NA	**8id**, KNI-10603, 87 (105)	NA	NA

The data are listed as the compound's referred name in bold, followed by its proprietary name, its experimental HTLV-I protease L40I mutant inhibition (nM, the largest standard-error-of-the-mean is 1.8 nM), and its predicted HTLV-I protease inhibition (nM) in parentheses as calculated from Eq. 1.
NA (not available): The compound was not synthesized.
Of the 44 compounds (**8aa–8id**), the HTLV-I and HIV-1 protease inhibitory activities of 10 compounds (**8ab**,**ba**,**bb**,**bc**,**cb**,**db**,**eb**,**fb**,**gb**,**hb**) have been previously reported.[21,22]

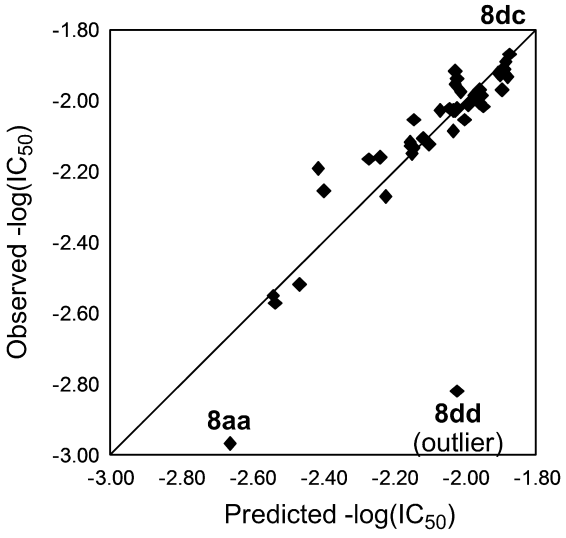


Figure 2. HTLV-I protease inhibition correlations between observed and predicted $-\log(IC_{50})$ values, based on Eq. 1, ranging from the least (**8aa**) to most (**8dc**) potent compound from 44 compounds, with compound **8dd** as an outlier.

centiles represented the score of the moiety being examined. To corroborate the scoring method, a well fitted, normalized, multiple collinear, QSAR equation (Eq. 1) was derived that associated HTLV-I protease inhibitory activity with the structures of the P_3-cap and P_1'-cap moieties. The equation is well fitted ($r^2 = 0.87$) and statistically significant ($p < 0.001$). Moreover, the equation was reliable because it consisted of only two parameters. A form of K-fold cross-validation, namely leave-one-out cross-validation, was performed using a single observation from the original sample as the validation data and the remaining observations as the training data, and the process was repeated such that each observation in the sample was used once as the validation data. In other words, many QSAR equations, in which one of the compounds was excluded, were derived to predict the HTLV-I protease inhibition of the excluded compound. Variations in the equations determined the validity of the overall equation, Eq. 1. The overall equation was valid, because the root-mean-square-deviations of the coefficients and intercept were relatively small (0.520 ± 0.014, 0.269 ± 0.008, -2.665 ± 0.015), and the coefficient of determination did not greatly vary during cross-validation ($r^2 = 0.85$–0.90). Cross-validation also confirmed the inhibitory activity of compound **8dd** as an outlier datum. We were unable to elucidate a reason for the lower HTLV-I protease inhibitory activity exhibited by

compound **8dd**. To account for the outlier, we synthesized compound **8id** as an analogue of compound **8dd**. We predicted that inhibitor **8id**'s IC_{50} value would be approximately 105 nM, which was the expected value for inhibitor **8dd**. Indeed, the experimental IC_{50} value of 87 nM was similar to the predicted value. Inhibitor **8id** was not used in deriving the QSAR equation due to the lack of comparable compounds.

In our most recent work, we suggested that the amide oxygen from the P_3-cap moiety was involved in a hydrogen bond interaction with Leu57A from the flap of the HTLV-I protease (Fig. 3C).[22] Indeed, the inhibitory activity data from the current work confirmed such an observation. Because of this important hydrogen bond interaction, the P_3-cap moiety contributed two-times more to the regression equation than the P'_1-cap moiety (P_3-cap moiety, 66%; P'_1-cap moiety, 34%). Interestingly, in an equation derived from a data set in which compounds lacking a P_3-cap moiety (**8aa**–**8af**) were excluded, the contribution to the equation was reversed, in that the P'_1-cap moiety contributed twice as much as the alkyl group on the P_3-cap moiety. The reason for such a reversal of equation contribution was elucidated from computer-assisted docking experiments that suggested that the P_3-cap moiety resided near the edge of the active site (Fig. 3B), whereas the P'_1-cap moiety was deeper within the active site (Fig. 3A). This implied that location-wise the hydrophobic region of the P_3-cap moiety was a lesser determinant of activity than that of the P'_1-cap moiety. From the scores derived for the P_3-cap moiety in Eq. 1, a statistically supported structure–activity trend suggested that lengthening alkyl group increased inhibitory potency against HTLV-I protease. Although not included in the derivation of Eq. 1, the potent HTLV-I protease inhibition profile of inhibitor **8id** also supported the observed alkyl-lengthening trend of the P_3-cap moiety. This observation agreed with our previously suggested trend that was derived from the inhibitory profiles of analogues containing an isobutylamino P'_1-cap moiety.[22] In the same former study, we also suggested methyl branching at either the P_3-cap moiety's α- or β-carbon increased inhibitory activity against HTLV-I protease. However, in contrast, the scores from the current and statistically relevant study suggested that increasing volumetric bulk at either the P_3-cap moiety's α- or β-carbon reduced HTLV-I protease inhibitory activity. We believe that the current study describes a more accurate trend. According to the overall scores, considering that lengthening the P_3-cap moiety enhanced HTLV-I protease inhibition whereas branching at the α- or β-carbon would diminish inhibitory activity, a flexible and non-bulky P_3-cap moiety would be more adequately accommodated by the protease.

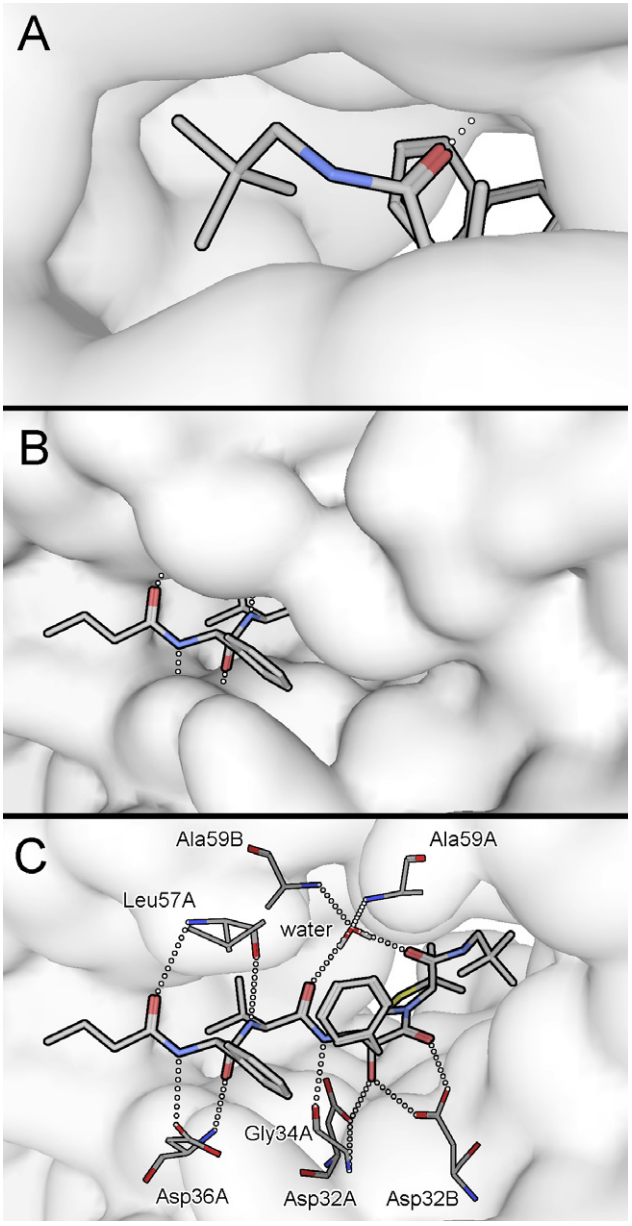


Figure 3. Computer model of KNI-10635 (**8dc**) in the active site of HTLV-I protease. Dotted lines represent possible hydrogen bond interactions. (A) The P'_1-cap moiety is located deeper within the active site than (B) the P_3 moiety which resides near the exposed surface of the HTLV-I protease. (C) The surface of some HTLV-I protease heavy atoms is hidden to clearly depict various hydrogen bond interactions.

According to the scores derived for the P'_1-cap moiety, increasing volumetric bulk at the P'_1-cap moiety's β-carbon enhanced HTLV-I protease inhibitory activity (Eq. 1). This observation agreed with our previous suggested trend that was based on the inhibitory activity values of a limited number of compounds.[21] Interestingly, the scores implied that methyl branching at the P'_1-cap moiety's (*R*)-α-carbon had an undesired effect on enzyme inhibition. Moreover, the scores also suggested that lengthening the P'_1-cap moiety also decreased inhibitory activity.

We were not compelled to complete the isopentyl P'_1-cap groups of compounds (e.g., inhibitors **8af**, **8df** and **8hf**). Synthesizing compounds **8bf**, **8cf**, **8ef**, **8ff** and **8gf** would only confirm that lengthening the P'_1-cap moiety reduced inhibitory activity against HTLV-I proteases, and we were more interested in designing more inhibitory active compounds. Moreover, we determined an ideal 'fit' within the enzyme for the P'_1-cap moiety's α- and β-carbon. The P'_1-cap moiety's γ-carbon would have less influence on binding than the α- and β-carbon, because the γ-carbon was relatively further away from the important catalytic S_1—S'_1 region. Note that we previously suggested that the hydrophobic region of the P_3-cap moiety had lesser influence on inhibitory activity than that of the P'_1-cap moiety, because the P_3-cap moiety was more distant from the S_1—S'_1 region than the P'_1-cap moiety.

Inhibitor **8dc** was predicted and observed as our more potent inhibitor described in the current study. To visualize and elucidate the correlations between inhibitory activity and chemical structure, we generated a computer-assisted docking model of inhibitor **8dc** in complex with an HTLV-I protease L40I mutant (Fig. 3). From the model, we observed various possible hydrogen bond interactions throughout the backbone of the inhibitor and the dimeric HTLV-I protease's Asp32A, Asp32B, Gly34A, Asp36A, Leu57A, Ala59A and Ala59B (Fig. 3C). A hydrogen bond network between Ala59A and Ala59B in the hairpin regions of HTLV-I protease's flaps and the inhibitor's P'_1 and P_2 amide oxygen was mediated by a water molecule in a similar fashion as that of HIV-1 and Plasmepsin protease inhibitors. Also in common, another hydrogen bond network was also formed between the oxygens of the inhibitor's P_1 hydroxymethylcarbonyl group and HTLV-I protease's catalytic

Asp32A and Asp32B, and thereby forming a transition-state mimic. Indeed, the P_3-cap moiety was accommodated near the edge of the active site region (Fig. 3B), whereas the location of the P'_1-cap moiety was deeper within the active site (Fig. 3A). Hence, the P'_1-cap moiety would have a greater location-wise influence than the P_3-cap moiety on the inhibitor's activity profile. It should however be noted that throughout the current study, we assumed that the P_3-cap and P'_1-cap moieties did not have any influence on each other. In reality, especially for the case of these small tetrapeptidic compounds, a slight structural change at one portion of the molecule would also affect the average orientation of other regions in the same molecule as well as regions of the HTLV-I protease.

Tözsér and co-workers reported narrower inhibition profiles from peptidic substrates for HTLV-I protease than HIV-1 protease.[17] In a similar pattern, we observed that HTLV-I protease expresses higher specificity than its HIV-1 protease cousin for the case inhibitors, in that all synthesized inhibitors exhibited almost complete HIV-1 protease inhibition at 50 nM ($\geqslant$98%). Indeed, the current work supported our past observations that, in general, HTLV-I protease inhibitors also exhibit extremely potent HIV-1 protease inhibition; however, potent HIV-1 protease inhibitors may not potently inhibit HTLV-I protease.[20–22] Hence, HTLV-I protease inhibitors might also provide a different design approach for novel HIV-1 protease inhibitors.

4. Conclusions

The current study described the design, synthesis and HTLV-I and HIV-1 protease inhibitory activity of 34 novel small tetrapeptidic inhibitors containing allophenylnorstatine as a substrate transition-state mimicking moiety. HTLV-I protease inhibitory activity was statistically correlated to the inhibitor's respective P_3- and P'_1-cap moieties. The IC_{50} value of the most potent inhibitor described in this study, KNI-10635 (**8dc**), was determined to be 73.8 ± 0.6 nM, and quantitative structure–activity relationship equations along with computer-assisted docking models were generated to explain the compound's highly potent inhibitory activity against HTLV-I protease.

5. Experimental

5.1. Chemistry

5.1.1. General methods

The detailed preparation of compounds **8ab**,**bb**,**cb**,**db**,**eb**,**fb**,**gb**,**hb** has already been reported.[22] Reagents and solvents were purchased from commercial suppliers and used without further purification. ^{1}H NMR spectra were measured on a JEOL JNM-AL300 FT (300 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard at ambient temperature. Data are reported as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, bs = broad singlet, m = multiplet) and number of protons. LC–MS was recorded on a Finnigan SSQ-7000 spectrometer. MALDI-TOF MS was performed on a Voyager-DE RP spectrometer and all target compounds were less than 1 *m/z* from calculated values. Preparative HPLC was obtained from a C_{18} reverse phase column (20 × 250 mm; YMC Pack ODS SH-343-5AM). Assay HPLC was performed on a C_{18} reverse phase column (3.0 × 75 mm; YMC Pack ODS AS-3E7). Analytical HPLC was performed using a C_{18} reverse phase column (4.6 × 150 mm; YMC Pack ODS AM-302) with a binary solvent system: linear gradient of CH_3CN in 0.1% aqueous trifluoroacetic acid (TFA) at a flow rate of 0.9 mL/min, detected at UV 230 nm. The purity of the desired compounds and intermediates was confirmed by analytical HPLC and was greater than 99% in two different systems (gradient: system A, 0–100%/40 min; system B, 30–90%/40 min).

5.1.2. General method A: amide bond formation

To a solution of substituted amine (1.0 mmol) in *N*,*N*-dimethyl formamide (DMF, 5 mL) were added Boc-Xaa-OH or carboxylic acid (1.1 mmol), BOP (1.1 mmol) and Et_3N (adjusted to pH 8), and stirred for 15 h at rt. After removal of the solution in vacuo, the residue was dissolved in EtOAc (20 mL), washed with 10% citric acid aq (3× 15mL), 10% $NaHCO_3$ aq (3× 15 mL) and brine (1× 15 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the crude product which was used directly to the next step without purification, or purified by preparative HPLC in the case of the target compounds. The purified target compounds were immediately lyophilized to afford their respective white amorphous powders.

5.1.3. General method B: Boc-deprotection

4 N HCl in 1,4-dioxane (5 mL) was added to the afforded crude Boc-protected product and stirred for 1 h at rt. The solution was evaporated under reduced pressure and used directly to the next step without purification. A small portion of the product was purified by preparative HPLC. The desired fractions were collected and immediately lyophilized to afford a white amorphous powder, compound **5**, **6**, or **7**.

5.1.3.1. (*R*)-*N*-Cyclopropylmethyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Dmt-NHCH$_2$^{c}Pr, 5a). Compound **5a** was prepared from Boc-Dmt-OH and aminomethylcyclopropane by general methods A and B. Yield: 98%; ^{1}H NMR ($CDCl_3$) δ = 0.20 (d, 2H), 0.50 (d, 2H), 0.90–1.02 (m, 1H), 1.43 (s, 3H), 1.65 (s, 3H), 3.09–3.24 (m, 2H), 4.33 (s, 1H), 4.45–4.60 (m, 2H), 6.22 (br s, 1H), 7.48–7.60 (m, 1H); MS (LC) *m/z* = 214.89 $[M+H]^+$; Analytical HPLC: t_R = 15.06 min (system A).

5.1.3.2. (*R*)-*N*-Cyclopropylmethyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenyl]butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Apns-Dmt-NHCH$_2$^{c}Pr, 6a). Compound **6a** was prepared from Boc-Apns-OH and compound by general methods A and B. Yield: 36%; ^{1}H NMR ($CDCl_3$) δ = 0.15 (d, 2H), 0.45 (d, 2H), 0.80–0.90 (m, 1H), 1.39–1.56 (m, 2× 3H), 2.7–3.1 (br s, 1H), 2.88–2.95 (m, 2H), 3.17–3.26 (m, 2H), 3.75–3.83 (br s, 1H), 4.21–4.39 (m, 1H+2H), 4.82 (d, 1H), 7.08–7.20 (m, 2H), 7.21–7.32 (m, 5H), 7.8–8.2 (br s, 1H); MS (TOF) *m/z* = 391.84 $[M+H]^+$, 414.007 $[M+Na]^+$; Analytical HPLC: t_R = 20.44 min (system A).

5.1.3.3. (*R*)-*N*-Cyclopropylmethyl-3-{(2*S*,3*S*)-3-[(2*S*)-2-amino-3,3-dimethyl]butanoylamino-2-hydroxy-4-phenyl}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Tle-Apns-Dmt-NHCH$_2$^{c}Pr, 7a). Compound **7a** was prepared from Boc-Tle-OH and compound **6a** by general methods A and B. Yield: 53%; ^{1}H NMR ($CDCl_3$) δ = 0.10–0.18 (m, 2H), 0.40–0.48 (m, 2H), 0.77–0.93 (m, 1H+3× 3H), 1.41–1.58 (m, 2× 3H), 1.7–2.5 (br s, 1H), 2.80–3.20 (m, 2× 2H), 3.51–3.64 (m, 1H), 4.29–4.40 (m, 1H), 4.48–4.84 (m, 1H+2H), 5.00–5.12 (m, 1H), 6.7–6.8 (m, 1H), 7.10–7.26 (m, 5H), 7.6–7.8 (br s, 1H), NH_2 not observed; MS (TOF) *m/z* = 504.425 $[M+H]^+$; Analytical HPLC: t_R = 21.67 min (system A).

5.1.3.4. (*R*)-*N*-Cyclopropylmethyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-amino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Phg-Tle-Apns-Dmt-NHCH$_2$^{c}Pr, 8aa). Compound **8aa** was prepared from Boc-Phg-OH and compound **7a** by general methods A and B. Yield: 41%; ^{1}H NMR ($CDCl_3$) δ = 0.14–0.20 (m, 2H), 0.40–0.48 (m, 2H), 0.64–0.73 (m, 3× 3H), 0.82–0.98 (m, 1H), 1.40–1.60 (m, 2× 3H), 1.6–2.0 (br s, 1H), 2.60–3.32 (m, 2× 2H), 3.83–4.35 (m, 2× 1H), 4.54–4.89 (m, 2× 1H+2H), 5.10–5.22 (m, 1H), 6.30–6.42 (m, 1H), 6.70–6.80 (m, 1H), 6.90–6.98 (m, 1H), 7.05–7.26 (m, 5H), 7.27–7.61 (m, 5H), NH_2 not observed; MS

(TOF) m/z = 638.654 $[M+H]^+$; Analytical HPLC: t_R = 23.14 min (system A), 9.60 min (system B).

5.1.3.5. (*R*)-*N*-Cyclopropylmethyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-acetylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (Ac-Phg-Tle-Apns-Dmt-NHCH$_2$cPr, 8ba). Compound **8ba** was prepared from acetic acid and compound **8aa** by general method A. Yield: 14%; ^{1}H NMR ($CDCl_3$) δ = 0.15–0.23 (m, 2H), 0.42–0.54 (m, 2H), 0.76–0.80 (m, 3× 3H), 0.88–0.98 (m, 1H), 1.47–1.59 (m, 2× 3H, partly covered by H_2O), 2.04–2.06 (m, 3H), 2.64–2.83 (m, 2H), 2.90–3.25 (m, 2H), 3.94–4.96 (m, 4× 1H+2H), 5.45 (t, 1H), 6.00 (d, 1H), 6.06 (d, 1H), 6.70 (d, 1H), 6.78 (d, 1H), 6.90–7.26 (m, 5H), 7.27–7.39 (m, 5H), OH not observed; MS (TOF) m/z = 680.806 $[M+H]^+$, 702.814 $[M+Na]^+$, 718.843 $[M+K]^+$; Analytical HPLC: t_R = 27.27 min (system A), 16.91 min (system B).

5.1.3.6. (*R*)-*N*-Cyclopropylmethyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-propionylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (EtCO-Phg-Tle-Apns-Dmt-NHCH$_2$^{c}Pr, 8ca). Compound **8ca** was prepared from propionic acid and compound **8aa** by general method A. Yield: 16%; ^{1}H NMR ($CDCl_3$) δ = 0.14 (d, 1H), 0.19 (d, 1H), 0.42 (d, 1H), 0.48 (d, 1H), 0.75–0.95 (m, 1H+3× 3H), 1.16 (t, 3H), 1.40–1.60 (m, 2× 3H), 2.30 (q, 2H), 2.6–3.2 (br s, 1H), 2.61–3.20 (m, 2× 2H), 3.95–4.99 (m, 4× 1H+2H), 5.51 (t, 1H), 6.15–6.25 (m, 1H), 6.30–6.45 (m, 1H), 6.70–6.80 (m, 1H), 6.81–6.89 (br s, 1H), 6.90–7.25 (m, 5H), 7.26–7.37 (m, 5H); MS (TOF) m/z = 695.001 $[M+H]^+$, 716.968 $[M+Na]^+$, 732.969 $[M+K]^+$; Analytical HPLC: t_R = 28.55 min (system A), 18.83 min (system B).

5.1.3.7. (*R*)-*N*-Cyclopropylmethyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-butyrylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{n}PrCO-Phg-Tle-Apns-Dmt-NHCH$_2$^{c}Pr, 8da). Compound **8da** prepared from *n*-butyric acid and compound **8aa** general method A. Yield: 15%; ^{1}H NMR ($CDCl_3$) δ = 0.15–0.24 (m, 2H), 0.41–0.54 (m, 2H), 0.75–0.82 (m, 3× 3H), 0.90–0.98 (m, 1H+3H), 1.47–1.73 (m, 2× 3H, partly covered by H_2O), 2.24 (t, 2H), 2.55–2.85 (m, 2H), 2.94–3.29 (m, 2H), 3.94–4.98 (m, 4× 1H+2H), 5.40–5.49 (m, 1H), 5.90–6.05 (m, 1H), 6.00 (d, 1H), 6.09 (d, 1H), 6.65–6.75 (m, 1H), 6.91–7.25 (m, 5H), 7.27–7.38 (m, 5H), OH not observed; MS (TOF) m/z = 730.888 $[M+Na]^+$, 746.916 $[M+K]^+$; Analytical HPLC: t_R = 30.03 min (system A), 20.38 min (system B).

5.1.3.8. (*R*)-*N*-Cyclopropylmethyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-cyclopropanecarbonylamino-2-phenyl]acetylamino-3,3-dimethyl}-butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{c}PrCO-Phg-Tle-Apns-Dmt-NHCH$_2$^{c}Pr, 8ea). Compound **8ea** was prepared from cyclopropanecarboxylic acid and compound 8aa by general method A. Yield: 13%; ^{1}H NMR ($CDCl_3$) δ = 0.11–0.21 (m, 2H), 0.40–0.51 (m, 2H), 0.74–1.05 (m, 1H+2× 2H+3× 3H), 1.45–1.60 (m, 1H+2× 3H), 1.7–2.1 (br s, 1H), 2.60–2.90 (m, 2H), 2.91–3.22 (m, 2H), 3.60–4.99 (m, 4× 1H+2H), 5.41–5.50 (m, 1H), 6.02–6.10 (m, 1H), 6.11–6.20 (m, 1H), 6.26 (d, 1H), 6.75 (t, 1H), 6.85–7.26 (m, 5H), 7.27–7.49 (m, 5H); MS (TOF) m/z = 728.893 $[M+Na]^+$, 744.72 $[M+K]^+$; Analytical HPLC: t_R = 29.02 min (system A), 20.24 min (system B).

5.1.3.9. (*R*)-*N*-Cyclopropylmethyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-isobutyrylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{i}PrCO-Phg-Tle-Apns-Dmt-NHCH$_2$^{c}Pr, 8fa). Compound **8fa** was prepared from isobutyric acid and compound **8aa** by general method A. Yield: 13%; ^{1}H NMR ($CDCl_3$) δ = 0.16–0.24 (m, 2H), 0.42–0.51 (m, 2H), 0.75–0.80 (m, 3× 3H), 0.85–0.97 (m, 1H), 1.10–1.22 (m, 2× 3H), 1.47–1.63 (m, 2× 3H), 1.8–2.2 (br s, 1H), 2.43–2.53 (m, 1H), 2.64–2.90 (m, 2H), 2.91–3.25 (m, 2H), 3.97–4.96 (m, 4× 1H+2H), 5.40–5.50 (m, 1H), 6.19 (d, 1H), 6.25 (d, 1H), 6.70–6.80 (m, 2× 1H), 6.90–7.27 (m, 5H), 7.28–7.42 (m, 5H); MS (TOF) m/z = 730.829 $[M+Na]^+$, 746.932 $[M+K]^+$; Analytical HPLC: t_R = 29.79 min (system A), 21.19 min (system B).

5.1.3.10. (*R*)-*N*-Cyclopropylmethyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-pivaloylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{t}BuCO-Phg-Tle-Apns-Dmt-NHCH$_2$^{c}Pr, 8ga). Compound **8ga** was prepared from pivalic acid and compound **8aa** by general method A. Yield: 11%; ^{1}H NMR ($CDCl_3$) δ = 0.10–0.24 (m, 2H), 0.35–0.52 (m, 2H), 0.79 (s, 3× 3H), 0.90–1.02 (m, 1H), 1.23 (s, 3× 3H), 1.47–1.60 (m, 2× 3H), 2.4–2.8 (br s, 1H), 2.64–2.85 (m, 2H), 2.88–3.25 (m, 2H), 3.98–5.04 (m, 4× 1H+2H), 5.43 (d, 1H), 6.20 (d, 1H), 6.28 (d, 1H), 6.78 (t, 1H), 6.87 (d, 1H), 6.92–7.25 (m, 5H), 7.26–7.37 (m, 5H); MS (TOF) m/z = 744.498 $[M+Na]^+$, 760.702 $[M+K]^+$; Analytical HPLC: t_R = 30.94 min (system A), 23.64 min (system B).

5.1.3.11. (*R*)-*N*-Cyclopropylmethyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-(3-methylbutanoyl)amino-2-phenyl]acetylamino-3,3-dimethyl}-butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{i}BuCO-Phg-Tle-Apns-Dmt-NHCH$_2$^{c}Pr, 8ha). Compound **8ha** was prepared from isovaleric acid and compound **8aa** by general method A. Yield: 17%; ^{1}H NMR ($CDCl_3$) δ = 0.14–0.24 (m, 2H), 0.40–0.52 (m, 2H), 0.75–0.80 (m, 3× 3H), 0.90–1.00 (m, 1H+2× 3H), 1.47–1.60 (m, 2× 3H), 1.8–2.2 (br s, 1H), 2.05–2.19 (m, 1H+2H), 2.58–2.93 (m, 2H), 2.94–3.29 (m, 2H), 3.97–4.99 (m, 4× 1H+2H), 5.43–5.51 (m, 1H), 6.09 (d, 1H), 6.16 (d, 1H), 6.70–6.80 (m, 2× 1H), 6.88–7.26 (m, 5H), 7.27–7.38 (m, 5H); MS (TOF) m/z = 745.088 $[M+Na]^+$, 761.321 $[M+K]^+$; Analytical HPLC: t_R = 30.03 min (system A), 22.60 min (system B).

5.1.3.12. (*R*)-*N*-(2,2-Dimethyl)propyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Dmt-NH^{t}Pe, 5c). Compound **5c** was prepared from 2,2-dimethylpropylamine and Boc-Dmt-OH by general methods A and B. Yield: 99%; ^{1}H NMR ($CDCl_3$) δ = 0.90 (s, 3× 3H), 1.43 (s, 3H), 1.65 (s, 3H), 3.03–3.20 (m, 2H), 4.35 (s, 1H), 4.52 (s, 2H), 6.2–6.5 (br s, 1H), 7.20–7.30 (m, 1H); MS (LC) m/z = 230.89 $[M+H]^+$, 252.89 $[M+Na]^+$; Analytical HPLC: t_R = 17.84 min (system A).

5.1.3.13. (*R*)-*N*-(2,2-Dimethyl)propyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenyl]butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Apns-Dmt-NH^{t}Pe, 6c). Compound **6c** was prepared from Boc-Apns-OH and compound **5c** by general methods A and B. Yield: 70%; ^{1}H NMR ($CDCl_3$) δ = 0.85–0.93 (m, 3× 3H), 1.38–1.54 (m, 2× 3H), 1.9–2.3 (br s, 1H), 2.90–3.25 (m, 2× 2H), 3.77–3.86 (m, 1H), 4.25–4.87 (m, 1H+2H), 4.73–4.85 (m, 1H), 6.85–6.95 (m, 1H), 7.13–7.20 (m, 2H), 7.26–7.35 (m, 5H); MS (TOF) m/z = 408.544 $[M+H]^+$; Analytical HPLC: t_R = 22.46 min (system A).

5.1.3.14. (*R*)-*N*-(2,2-Dimethyl)propyl-3-{(2*S*,3*S*)-3-[(2*S*)-2-amino-3,3-dimethyl]butanoylamino-2-hydroxy-4-phenyl}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Tle-Apns-Dmt-NHCH$_2$^{t}Bu, 7c). Compound **7c** was prepared from Boc-Tle-OH and compound **6c** by general methods A and B. Yield: 85%; ^{1}H NMR ($CDCl_3$) δ = 0.80–0.93 (m, 6× 3H), 1.43–1.52 (m, 2× 3H), 2.1–2.5 (br s, 1H), 2.80–3.20 (m, 2× 2H), 3.50–3.68 (m, 1H), 4.31–5.12 (m, 3× 1H+2H), 6.4–6.7 (br s, 1H), 7.06–7.29 (m, 5H),

7.8–8.0 (br s, 1H), NH_2 not observed; MS (TOF) m/z = 521.376 $[M+H]^+$. Analytical HPLC: t_R = 23.10 min (system A).

5.1.3.15. (*R*)-*N*-(2,2-Dimethyl)propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-amino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Phg-Tle-Apns-Dmt-NH^{t}Pe, 8ac). Compound **8ac** was prepared from Boc-Phg-OH and compound **8ac** by general methods A and B. Yield: 57%. ^{1}H NMR ($CDCl_3$) δ = 0.72–0.82 (m, 3× 3H), 0.83–0.95 (m, 3× 3H), 1.44–1.60 (m, 2× 3H), 2.07 (s, 3H), 2.5–2.9 (br s, 1H), 2.65–3.26 (m, 2× 2H), 3.97–4.99 (m, 4× 1H+2H), 5.53–5.60 (m, 1H), 6.15 (br s, 1H), 6.33 (br s, 1H), 6.46 (d, 1H), 6.68 (t, 1H), 6.90–7.24 (m, 5H), 7.28–7.38 (m, 5H). MS (TOF) m/z = 696.772 $[M+H]^+$, 718.777 $[M+Na]^+$, 734.805 $[M+K]^+$. Analytical HPLC: t_R = 29.62 min (system A), 20.98 min (system B).

5.1.3.16. (*R*)-*N*-(2,2-Dimethyl)propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-acetylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (Ac-Phg-Tle-Apns-Dmt-NH^{t}Pe, 8bc). Compound **8bc** was prepared from acetic acid and compound **8ac** by general method A. Yield: 57%. ^{1}H NMR ($CDCl_3$) δ = 0.72–0.82 (m, 3× 3H), 0.83–0.95 (m, 3× 3H), 1.44–1.60 (m, 2× 3H), 2.07 (s, 3H), 2.5–2.9 (br s, 1H), 2.65–3.26 (m, 2× 2H), 3.97–4.99 (m, 4× 1H+2H), 5.53–5.60 (m, 1H), 6.15 (br s, 1H), 6.33 (br s, 1H), 6.46 (d, 1H), 6.68 (t, 1H), 6.90–7.24 (m, 5H), 7.28–7.38 (m, 5H). MS (TOF) m/z = 696.772 $[M+H]^+$, 718.777 $[M+Na]^+$, 734.805 $[M+K]^+$. Analytical HPLC: t_R = 29.62 min (system A), 20.98 min (system B).

5.1.3.17. (*R*)-*N*-(2,2-Dimethyl)propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-propionylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (EtCO-Phg-Tle-Apns-Dmt-NH^{t}Pe, 8cc). Compound **8cc** was prepared from propionic acid and compound **8ac** by general method A. Yield: 58%. ^{1}H NMR ($CDCl_3$) δ = 0.75–0.80 (m, 3× 3H), 0.81–0.91 (m, 3× 3H), 1.16 (t, 3H), 1.47–1.59 (m, 2× 3H), 2.2–2.5 (br s, 1H), 2.24–2.36 (m, 2H), 2.60–3.29 (m, 2× 2H), 3.97–5.01 (m, 4× 1H+2H), 5.51 (d, 1H), 6.17 (d, 1H), 6.27 (d, 1H), 6.69 (t, 1H), 6.84 (d, 1H), 6.90–7.23 (m, 5H), 7.28–7.38 (m, 5H). MS (TOF) m/z = 732.917 $[M+Na]^+$, 748.958 $[M+K]^+$. Analytical HPLC: t_R = 30.59 min (system A), 22.63 min (system B).

5.1.3.18. (*R*)-*N*-(2,2-Dimethyl)propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-butyrylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{n}PrCO-Phg-Tle-Apns-Dmt-NH^{t}Pe, 8dc). Compound **8dc** was prepared from *n*-butyric acid and compound **8ac** by general method A. Yield: 63%. ^{1}H NMR ($CDCl_3$) δ = 0.75–0.85 (m, 3× 3H), 0.86–0.96 (m, 4× 3H), 1.4–2.0 (br s, 1H), 1.49–1.59 (m, 2× 3H), 1.63–1.70 (m, 2H), 2.20–2.29 (m, 2H), 2.55–2.80 (m, 2H), 2.84–3.30 (m, 2H), 3.95–4.99 (m, 4× 1H+2H), 5.50 (d, 1H), 6.10 (d, 1H), 6.18 (d, 1H), 6.68 (t, 1H), 6.78 (d, 1H), 6.90–7.25 (m, 5H), 7.27–7.40 (m, 5H). MS (TOF) m/z = 725.065 $[M+H]^+$, 747.012 $[M+Na]^+$, 763.009 $[M+K]^+$. Analytical HPLC: t_R = 32.06 min (system A), 24.40 min (system B).

5.1.3.19. (*R*)-*N*-(2,2-Dimethyl)propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-cyclopropanecarbonylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{c}PrCO-Phg-Tle-Apns-Dmt-NH^{t}Pe, 8ec). Compound **8ec** was prepared from cyclopropanecarboxylic acid and compound **8ac** by general method A. Yield: 60%. ^{1}H NMR ($CDCl_3$) δ = 0.76–0.99 (m, 2× 2H+6× 3H), 1.47–1.57 (m, 1H+2× 3H), 2.5–3.0 (br s, 1H), 2.58–3.29 (m, 2× 2H), 4.02–5.04 (m, 4× 1H+2H), 5.54 (d, 1H), 5.61 (d, 1H), 6.32 (d, 1H), 6.55 (d, 1H), 6.69 (t, 1H), 6.91–7.24 (m, 5H), 7.26–7.38 (m, 5H). MS (TOF) m/z = 744.939 $[M+Na]^+$, 760.936 $[M+K]^+$. Analytical HPLC: t_R = 31.76 min (system A), 23.95 min (system B).

5.1.3.20. (*R*)-*N*-(2,2-Dimethyl)propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-isobutyrylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{i}PrCO-Phg-Tle-Apns-Dmt-NH^{t}Pe, 8fc). Compound **8fc** was prepared from isobutyric acid and compound **8ac** by general method A. Yield: 62%. ^{1}H NMR ($CDCl_3$) δ = 0.74–0.96 (m, 8× 3H), 1.4–1.8 (br s, 1H), 1.45–1.60 (m, 2× 3H), 2.05–2.14 (m, 1H+2H), 2.61–2.80 (m, 2H), 2.83–3.29 (m, 2H), 3.93–4.99 (m, 4× 1H+2H), 5.45–5.50 (m, 1H), 6.01 (d, 1H), 6.09 (d, 1H), 6.68 (t, 1H), 6.74 (d, 1H), 6.85–7.26 (m, 5H), 7.27–7.40 (m, 5H). MS (TOF) m/z = 761.022 $[M+Na]^+$, 777.014 $[M+K]^+$. Analytical HPLC: t_R = 33.07 min (system A), 25.92 min (system B).

5.1.3.21. (*R*)-*N*-(2,2-Dimethyl)propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-pivaloylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{t}BuCO-Phg-Tle-Apns-Dmt-NH^{t}Pe, 8gc). Compound **8gc** was prepared from pivalic acid and compound **8ac** by general method A. Yield: 59%. ^{1}H NMR ($CDCl_3$) δ = 0.75–0.98 (m, 6× 3H), 1.12–1.29 (m, 3× 3H), 1.47–1.60 (m, 2× 3H), 2.6–3.0 (br s, 1H), 2.60–3.29 (m, 2× 2H), 3.97–5.05 (m, 4× 1H+2H), 5.45 (d, 1H), 5.50 (d, 1H), 6.19 (d, 1H), 6.24 (d, 1H), 6.70 (t, 1H), 6.91–7.25 (m, 5H), 7.26–7.40 (m, 5H). MS (TOF) m/z = 760.994 $[M+Na]^+$, 776.945 $[M+K]^+$. Analytical HPLC: t_R = 33.66 min (system A), 27.15 min (system B).

5.1.3.22. (*R*)-*N*-(2,2-Dimethyl)propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-(3-methyl)butanoylamino-2-phenyl]acetylamino-3,3-dimethyl}-butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{i}BuCO-Phg-Tle-Apns-Dmt-NH^{t}Pe, 8hc). Compound **8hc** was prepared from isovaleric acid and compound **8ac** by general method A. Yield: 60%. ^{1}H NMR ($CDCl_3$) δ = 0.75–0.80 (m, 3× 3H), 0.82–0.93 (m, 3× 3H), 1.12–1.21 (m, 2× 3H), 1.4–1.9 (br s, 1H), 1.49–1.59 (m, 2× 3H), 2.42–2.50 (m, 1H), 2.56–2.80 (m, 2H), 2.81–3.31 (m, 2H), 3.94–5.00 (m, 4× 1H+2H), 5.42–5.48 (m, 1H), 6.08 (d, 1H), 6.17 (d, 1H), 6.70 (t, 1H), 6.72 (d, 1H), 6.90–7.26 (m, 5H), 7.28–7.39 (m, 5H). MS (TOF) m/z = 725.082 $[M+H]^+$, 747.071 $[M+Na]^+$, 763.032 $[M+K]^+$. Analytical HPLC: t_R = 32.16 min (system A), 27.39 min (system B).

5.1.3.23. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [H-Dmt-NHCH(Me)^{i}Pr, 5d]. Compound **5d** was prepared from (*R*)-3-methyl-2-butylamine and Boc-Dmt-OH by general methods A and B. Yield: 98%. ^{1}H NMR ($CDCl_3$) δ = 0.89 (d, 2× 3H), 1.00 (d, 3H), 1.41 (s, 3H), 1.62–1.74 (m, 1H+3H), 3.2–3.6 (br s, 1H), 3.75–3.90 (m, 1H), 4.25 (s, 1H), 4.48 (s, 2H), 6.86 (d, 1H). MS (LC) m/z = 230.89 $[M+H]^+$. Analytical HPLC: t_R = 17.38 min (system A).

5.1.3.24. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenyl]butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [H-Apns-Dmt-NHCH(Me)^{i}Pr, 6d]. Compound **6d** was prepared from compound **5d** and Boc-Apns-OH by general methods A and B. Yield: 43%. ^{1}H NMR ($CDCl_3$) δ = 0.80–0.95 (m, 2× 3H), 1.00–1.10 (m, 3H), 1.39–1.51 (m, 2× 3H), 1.63–1.74 (m, 1H), 1.8–2.2 (br s, 1H), 2.90–3.22 (m, 2H), 3.70–3.90 (m, 2× 1H), 4.25–4.86 (m, 2× 1H+2H), 6.65–6.75 (m, 1H), 7.20–7.33 (m, 5H). NH_2 not observed. MS (TOF) m/z = 407.926 $[M+H]^+$. Analytical HPLC: t_R = 22.55 min (system A).

5.1.3.25. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{(2*S*,3*S*)-3-[(2*S*)-2-amino-3,3-dimethyl]butanoylamino-2-hydroxy-4-phenyl}-butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [H-Tle-Apns-Dmt-NHCH(Me)^{i}Pr, 7d]. Compound **7d** was prepared from Boc-Tle-OH and compound **6d** by general methods A and B. Yield: 64%. ^{1}H NMR ($CDCl_3$) δ = 0.75–1.05 (m, 6× 3H), 1.30–1.52 (m, 2× 3H), 1.60–1.71 (m, 1H), 2.0–2.4 (br s, 1H), 2.80–3.15 (m, 2H), 3.50–3.83 (m, 2× 1H), 4.25–5.12 (m, 3× 1H+2H), 6.05–6.30 (m, 1H), 7.10–7.26 (m, 5H), 7.75–7.92 (m, 1H). NH_2 not observed. MS (TOF) m/z = 520.486 $[M+H]^+$, 543.446 $[M+Na]^+$, 559.196 $[M+K]^+$. Analytical HPLC: t_R = 22.71 min (system A).

5.1.3.26. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-amino-2-phenyl]acetylamino-3,3-dimethyl} butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [H-Phg-Tle-Apns-Dmt-NHCH(Me)^{i}Pr, 8ad]. Compound **8ad** was prepared from Boc-Phg-OH and compound **7d** and by general methods A and B. Yield: 63%. ^{1}H NMR ($CDCl_3$) δ = 0.55–0.80 (m, 3× 3H), 0.82–1.05 (m, 3× 3H), 1.40–1.56 (m, 2× 3H), 1.60–1.70 (m, 1H), 1.8–2.3 (br s, 1H), 2.50–2.85 (m, 2H), 3.72–4.87 (m, 5× 1H+2H), 5.15–5.40 (m, 1H), 6.27–6.38 (m, 1H), 6.70–6.85 (m, 1H), 6.90–6.98 (m, 1H), 7.10–7.26 (m, 5H), 7.27–7.58 (m, 5H). NH_2 not observed. MS (TOF) m/z = 676.939 $[M+Na]^+$. Analytical HPLC: t_R = 24.99 min (system A), 13.42 min (system B).

5.1.3.27. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-acetylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoyl-amino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [Ac-Phg-Tle-Apns-Dmt-NHCH(Me)^{i}Pr, 8bd]. Compound **8bd** was prepared from acetic acid and compound **8ad** by general method A. Yield: 33%. ^{1}H NMR ($CDCl_3$) δ = 0.72–0.79 (m, 3× 3H), 0.85–0.92 (m, 2× 3H), 0.99–1.07 (m, 3H), 1.45–1.73 (m, 1H+2× 3H, partly covered by H_2O), 2.00–2.07 (m, 3H), 2.53–2.77 (m, 2H), 3.77–5.00 (m, 5× 1H+2H), 5.47 (t, 1H), 5.63 (d, 1H), 6.09 (t, 1H), 6.32 (d, 1H), 6.80 (br s, 1H), 6.85–7.26 (m, 5H), 7.30–7.40 (m, 5H). OH not observed. MS (TOF) m/z = 696.859 $[M+H]^+$, 718.847 $[M+Na]^+$, 734.898 $[M+K]^+$. Analytical HPLC: t_R = 28.35 min (system A), 20.08 min (system B).

5.1.3.28. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-propionylamino-2-phenyl]acetylamino-3,3-dimethyl}-butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [EtCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{i}Pr, 8cd]. Compound **8cd** was prepared from propionic acid and compound **8ad** by general method A. Yield: 33%. ^{1}H NMR ($CDCl_3$) δ = 0.70–0.80 (m, 3× 3H), 0.83–0.95 (m, 2× 3H), 0.97–1.07 (m, 3H), 1.16 (t, 3H), 1.46–1.59 (m, 2× 3H), 1.5–2.0 (br s, 1H), 1.63–1.78 (m, 1H), 2.30 (q, 2H), 2.60–3.00 (m, 2H), 3.65–5.00 (m, 5× 1H+2H), 5.41–5.48 (m, 1H), 5.65 (d, 1H), 6.05 (d, 1H), 6.30 (d, 1H), 6.75 (d, 1H), 6.83–7.25 (m, 5H), 7.27–7.39 (m, 5H). MS (TOF) m/z = 732.826 $[M+Na]^+$, 748.834 $[M+K]^+$. Analytical HPLC: t_R = 31.31 min (system A), 21.79 min (system B).

5.1.3.29. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-butyrylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{n}PrCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{i}Pr, 8dd]. Compound **8dd** was prepared from *n*-butyric acid and compound **8ad** by general method A. Yield: 32%. ^{1}H NMR ($CDCl_3$) δ = 0.72–0.78 (m, 3× 3H), 0.80–1.07 (m, 4× 3H), 1.46–1.70 (m, 1H+2H+2× 3H), 1.5–1.9 (br s, 1H), 2.24 (t, 2H), 2.56–2.79 (m, 2H), 3.65–5.00 (m, 5× 1H+2H), 5.41–5.49 (m, 1H), 5.68 (d, 1H), 6.10 (d, 1H), 6.30 (d, 1H), 6.75 (d, 1H), 6.87–7.25 (m, 5H), 7.26–7.40 (m, 5H). MS (TOF) m/z = 724.896 $[M+H]^+$, 746.818 $[M+Na]^+$, 762.872 $[M+K]^+$. Analytical HPLC: t_R = 32.00 min (system A), 23.19 min (system B).

5.1.3.30. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-cyclopropanecarbonylamino-2-phenyl]acetylamino-3,-3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{c}PrCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{i}Pr, 8ed]. Compound **8ed** was prepared from cyclopropanecarboxylic acid and compound **8ad** by general method A. Yield: 32%. ^{1}H NMR ($CDCl_3$) δ = 0.75–0.80 (m, 3× 3H), 0.82–0.92 (m, 3× 3H), 0.95–1.08 (m, 2× 2H), 1.46–1.73 (m, 2× 1H+2× 3H, partly covered by H_2O), 2.54–2.80 (m, 2H), 3.80–5.02 (m, 5× 1H+2H), 5.40–5.49 (m, 1H), 5.68 (d, 1H), 6.06 (br s, 1H), 6.20 (d, 1H), 6.36 (d, 1H), 6.88–7.26 (m, 5H), 7.30–7.42 (m, 5H). OH not observed. MS (TOF) m/z = 723.101 $[M+H]^+$, 745.055 $[M+Na]^+$, 761.039 $[M+K]^+$. Analytical HPLC: t_R = 31.18 min (system A), 23.04 min (system B).

5.1.3.31. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-isobutyrylamino-2-phenyl] acetylamino-3,3-dimethyl} butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{i}PrCO-Phg-Tle-Apns-Dmt-NHCH (Me)^{i}Pr, 8fd]. Compound **8fd** was prepared from isobutyric acid and compound **8ad** by general method A. Yield: 35%. ^{1}H NMR ($CDCl_3$) δ = 0.75–1.20 (m, 8× 3H), 1.46–1.75 (m, 1H+2× 3H), 1.9–2.3 (br s, 1H), 2.40–2.50 (m, 1H), 2.58–2.99 (m, 2H), 3.73–5.00 (m, 5× 1H+2H), 5.38–5.47 (m, 1H), 5.71 (d, 1H), 6.15 (d, 1H), 6.30 (d, 1H), 6.75 (d, 1H), 6.88–7.25 (m, 5H), 7.26–7.41 (m, 5H). MS (TOF) m/z = 746.58 $[M+Na]^+$, 762.56 $[M+K]^+$. Analytical HPLC: t_R = 31.58 min (system A), 24.24 min (system B).

5.1.3.32. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-pivaloylamino-2-phenyl]acetylamino-3,3-dimethyl}-butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{t}BuCO-Phg-Tle-Apns-Dmt-NHCH (Me)^{i}Pr, 8gd]. Compound **8gd** was prepared from pivalic acid and compound **8ad** by general method A. Yield: 34%. ^{1}H NMR ($CDCl_3$) δ = 0.66–0.79 (m, 3× 3H), 0.84–0.98 (m, 2× 3H), 1.00–1.09 (m, 3H), 1.20–1.23 (m, 3× 3H), 1.45–1.73 (m, 1H+2× 3H, partly covered by H_2O), 2.57–2.83 (m, 2H), 3.69–5.03 (m, 5× 1H+2H), 5.30–5.41 (m, 1H), 5.61–5.69 (m, 1H), 6.00–6.13 (m, 1H), 6.27–6.38 (m, 1H), 6.83–6.93 (m, 1H), 7.03–7.26 (m, 5H), 7.27–7.40 (m, 5H). OH not observed. MS (TOF) m/z = 761.084 $[M+Na]^+$, 777.135 $[M+K]^+$. Analytical HPLC: t_R = 32.99 min (system A), 26.71 min (system B).

5.1.3.33. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-(3-methylbutanoyl)amino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{t}BuCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{i}Pr, 8hd]. Compound **8hd** was prepared from isovaleric acid and compound **8ad** by general method A. Yield: 30%. ^{1}H NMR ($CDCl_3$) δ = 0.70–1.07 (m, 8× 3H), 1.46–1.68 (m, 1H+2× 3H, partly covered by H_2O), 2.06–2.13 (m, 1H+2H), 2.54–2.79 (m, 2H), 3.75–5.00 (m, 5× 1H+2H), 5.42–5.48 (m, 1H), 5.60 (d, 1H), 5.90 (d, 1H), 6.28 (d, 1H), 6.68 (d, 1H), 6.88–7.26 (m, 5H), 7.27–7.41 (m, 5H). OH not observed. MS (TOF) m/z = 761.194 $[M+Na]^+$, 777.078 $[M+K]^+$. Analytical HPLC: t_R = 31.82 min (system A), 25.07 min (system B).

5.1.3.34. (*R*)-*N*-[(*R*)-1,2-Dimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-(4-pentenoyl)amino-2-phenyl]acetylamino-3,3-dimethyl}-butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [CH_2=$CH(CH_2)_2$CO-Phg-Tle-Apns-Dmt-NHCH(Me)^{i}Pr, 8id]. Compound **8id** was prepared from 4-pentenoic acid and compound **8ad** by general method A. Yield:

30%. ^{1}H NMR ($CDCl_3$) δ = 0.70–1.09 (m, 6× 3H), 1.35–1.60 (m, 2× 3H), 1.62–1.73 (m, 1H), 1.8–2.2 (br s, 1H), 2.26–2.40 (m, 2× 2H), 2.52–2.80 (m, 2H), 3.75–5.09 (m, 5× 1H+2× 2H), 5.35–5.85 (m, 2× 1H), 6.03–6.09 (m, 1H), 6.10–6.20 (m, 1H), 6.28–6.33 (m, 1H), 6.75–6.93 (m, 1H), 7.03–7.25 (m, 5H), 7.26–7.40 (m, 5H). MS (TOF) m/z = 758.894 $[M+Na]^+$, 774.911 $[M+K]^+$. Analytical HPLC: t_R = 32.08 min (system A), 25.28 min (system B).

5.1.3.35. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [H-Dmt-NHCH(Me)^{t}Bu, 5e]. Compound **5e** was prepared from Boc-Dmt-OH and (*R*)-1,2,2-trimethylpropylamine by general methods A and B. Yield: 98%. ^{1}H NMR ($CDCl_3$) δ = 0.89 (s, 3× 3H), 1.08 (d, 3H), 1.42 (s, 3H), 1.63 (s, 3H), 3.86–3.95 (m, 1H), 4.33 (s, 1H), 4.51 (s, 2H), 5.0–5.4 (br s, 1H), 6.85 (d, 1H). MS (LC) m/z = 245.40 $[M+H]^+$. Analytical HPLC: t_R = 18.54 min (system A).

5.1.3.36. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenyl]butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [H-Apns-Dmt-NH NHCH(Me)^{t}Bu, 6e]. Compound **6e** was prepared from Boc-Apns-OH and compound **5e** by general methods A and B. Yield: 45%. ^{1}H NMR ($CDCl_3$) δ = 0.85–0.95 (m, 3× 3H), 0.98–1.09 (m, 3H), 1.35–1.51 (m, 2× 3H), 2.3–2.8 (br s, 1H), 2.61–2.94 (m, 2H), 3.06–3.25 (m, 1H), 3.81–3.92 (m, 1H), 4.30–4.54 (m, 1H+2H), 4.71–4.88 (m, 1H), 6.65–6.75 (m, 1H), 7.26–7.37 (m, 5H), 7.9–8.3 (br s, 2H). MS (TOF) m/z = 421.477 $[M+H]^+$. Analytical HPLC: t_R = 23.16 min (system A).

5.1.3.37. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-{(2*S*,3*S*)-3-[(2*S*)-2-amino-3,3-dimethyl]butanoylamino-2-hydroxy-4-phenyl}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [H-Tle-Apns-Dmt-NHCH(Me)^{t}Bu, 7e]. Compound **7e** was prepared from Boc-Tle-OH and compound **6e** by general methods A and B. Yield: 62%. ^{1}H NMR ($CDCl_3$) δ = 0.80–1.15 (m, 7× 3H), 1.35–1.60 (m, 2× 3H), 2.8–4.0 (br s, 1H), 2.80–3.20 (m, 2H), 3.40–3.60 (m, 1H), 3.70–3.85 (m, 1H), 4.25–5.14 (m, 3× 1H+2H), 5.95–6.05 (m, 1H), 6.15–6.25 (m, 1H), 7.15–7.26 (m, 5H), 7.5–7.7 (br s, 2H). MS (TOF) m/z = 535.28 $[M+H]^+$. Analytical HPLC: t_R = 23.95 min (system A), 11.46 min (system B).

5.1.3.38. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-amino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [H-Phg-Tle-Apns-Dmt-NHCH(Me)^{t}Bu, 8ae]. Compound **8ae** was prepared from Boc-Phg-OH and compound **7e** by general methods A and B. Yield: 65%. ^{1}H NMR ($CDCl_3$) δ = 0.60–0.73 (m, 3× 3H), 0.81–1.03 (m, 4× 3H), 1.40–1.55 (m, 2× 3H), 2.4–2.8 (br s, 1H), 2.53–2.80 (m, 2H), 3.63–4.95 (m, 5× 1H+2H), 5.22–5.41 (m, 1H), 6.50–6.65 (m, 1H), 6.70–6.80 (m, 1H), 6.83–6.93 (m, 1H), 7.05–7.20 (m, 5H), 7.37–7.60 (m, 5H). NH_2 not observed. MS (TOF) m/z = 668.096 $[M+H]^+$, 690.374 $[M+Na]^+$, 706.47 $[M+K]^+$. Analytical HPLC: t_R = 25.99 min (system A), 15.63 min (system B).

5.1.3.39. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-acetylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [Ac-Phg-Tle-Apns-Dmt-NHCH(Me)^{t}Bu, 8be]. Compound **8be** was prepared from acetic acid and compound **8ae** by general method A. Yield: 32%. ^{1}H NMR ($CDCl_3$) δ = 0.75–0.80 (m, 3× 3H), 0.85–0.93 (m, 3× 3H), 1.00–1.06 (m, 3H), 1.45–1.64 (m, 2× 3H, partly covered by H_2O), 2.03–2.06 (m, 3H), 2.50–2.77 (m, 2H), 3.79–5.06 (m, 5× 1H+2H), 5.45–5.50 (m, 1H), 5.59 (d, 1H), 5.98 (d, 1H), 6.09 (d, 1H), 6.28 (d, 1H), 6.78–7.25 (m, 5H), 7.29–7.42 (m, 5H). OH not observed. MS (TOF) m/z = 710.872 $[M+H]^+$, 732.927 $[M+Na]^+$, 748.945 $[M+K]^+$. Analytical HPLC: t_R = 30.30 min (system A), 22.23 min (system B).

5.1.3.40. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-propionylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [EtCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{t}Bu, 8ce]. Compound **8ce** was prepared from propionic acid and compound **8ae** by general method A. Yield: 35%. ^{1}H NMR ($CDCl_3$) δ = 0.74–0.78 (m, 3× 3H), 0.88–0.92 (m, 3× 3H), 1.00–1.06 (m, 3H), 1.10–1.18 (m, 3H), 1.45–1.60 (m, 2× 3H, partly covered by H_2O), 2.23–2.38 (m, 2H), 2.50–2.79 (m, 2H), 3.80–5.17 (m, 5× 1H+2H), 5.41–5.47 (m, 1H), 5.52 (d, 1H), 6.03 (d, 1H), 6.11 (d, 1H), 6.24 (d, 1H), 6.70–7.26 (m, 5H), 7.27–7.43 (m, 5H). OH not observed. MS (TOF) m/z = 746.875 $[M+Na]^+$, 762.813 $[M+K]^+$. Analytical HPLC: t_R = 31.26 min (system A), 23.75 min (system B).

5.1.3.41. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-butyrylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{n}PrCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{t}Bu, 8de]. Compound **8de** was prepared from *n*-butyric acid and compound **8ae** by general method A. Yield: 31%. ^{1}H NMR ($CDCl_3$) δ = 0.73–0.78 (m, 3× 3H), 0.85–0.95 (m, 4× 3H), 0.96–1.07 (m, 3H), 1.45–1.70 (m, 2H+2× 3H), 1.5–1.8 (br s, 1H), 2.18–2.29 (m, 2H), 2.53–2.73 (m, 2H), 3.78–5.05 (m, 5× 1H+2H), 5.46–5.50 (m, 1H), 5.65 (d, 1H), 6.01 (d, 1H), 6.19 (d, 1H), 6.26 (d, 1H), 6.70–7.24 (m, 5H), 7.28–7.42 (m, 5H). MS (TOF) m/z = 760.828 $[M+Na]^+$, 776.946 $[M+K]^+$. Analytical HPLC: t_R = 32.70 min (system A), 26.80 min (system B).

5.1.3.42. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-cyclopropanecarbonylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{c}PrCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{t}Bu, 8ee]. Compound **8ee** was prepared from cyclopropanecarboxylic acid and compound **8ae** by general method A. Yield: 33%. ^{1}H NMR ($CDCl_3$) δ = 0.71–1.07 (m, 2× 2H+7× 3H), 1.45–1.58 (m, 1H+2× 3H), 1.6–2.0 (br s, 1H), 2.60–2.77 (m, 2H), 3.79–5.08 (m, 5× 1H+2H), 5.47 (t, 1H), 5.63 (d, 1H), 6.12 (d, 1H), 6.19 (d, 1H), 6.28 (d, 1H), 6.85–7.25 (m, 5H), 7.27–7.40 (m, 5H). MS (TOF) m/z = 758.873 $[M+Na]^+$, 774.895 $[M+K]^+$. Analytical HPLC: t_R = 32.08 min (system A), 25.10 min (system B).

5.1.3.43. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-isobutyrylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{i}PrCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{t}Bu, 8fe]. Compound **8fe** was prepared from isobutyric acid and compound **8ae** by general method A. Yield: 33%. ^{1}H NMR ($CDCl_3$) δ = 0.71–1.07 (m, 2× 2H+7× 3H), 1.45–1.58 (m, 1H+2× 3H), 1.6–2.0 (br s, 1H), 2.60–2.77 (m, 2H), 3.79–5.08 (m, 5× 1H+2H), 5.47 (t, 1H), 5.63 (d, 1H), 6.12 (d, 1H), 6.19 (d, 1H), 6.28 (d, 1H), 6.85–7.25 (m, 5H), 7.27–7.40 (m, 5H). MS (TOF) m/z = 758.873 $[M+Na]^+$, 774.895 $[M+K]^+$. Analytical HPLC: t_R = 32.08 min (system A), 25.10 min (system B).

5.1.3.44. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-pivaloylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{t}BuCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{t}Bu, 8ge]. Compound **8ge** was prepared from pivalic acid and compound **8ae** by general method A. Yield: 32%. ^{1}H NMR ($CDCl_3$) δ = 0.72–0.78 (m, 3× 3H), 0.85–0.90 (m, 3× 3H), 0.97–1.06 (m, 3H), 1.22 (s, 3× 3H), 1.47–1.57 (m, 2×

3H), 1.7–2.0 (br s, 1H), 2.50–2.73 (m, 2H), 3.77–5.10 (m, 5× 1H+2H), 5.35–5.45 (m, 1H), 5.64 (d, 1H), 6.05 (d, 1H), 6.18 (d, 1H), 6.26 (d, 1H), 6.85–7.19 (m, 5H), 7.26–7.42 (m, 5H). MS (TOF) m/z = 775.167 $[M+Na]^+$, 791.149 $[M+K]^+$. Analytical HPLC: t_R = 34.16 min (system A), 28.38 min (system B).

5.1.3.45. (*R*)-*N*-[(*R*)-1,2,2-Trimethyl]propyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-(3-methylbutanoyl)amino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide [^{i}BuCO-Phg-Tle-Apns-Dmt-NHCH(Me)^{t}Bu, 8he]. Compound **8he** was prepared from isovaleric acid and compound **8ae** by general method A. Yield: 31%. ^{1}H NMR ($CDCl_3$) δ = 0.70–1.04 (m, 9× 3H), 1.45–1.55 (m, 2× 3H), 1.7–2.2 (br s, 1H), 2.00–2.20 (m, 1H+2H), 2.52–2.78 (m, 2H), 3.75–5.07 (m, 5× 1H+2H), 5.51–5.59 (m, 1H), 5.83 (d, 1H), 6.13 (d, 1H), 6.27 (d, 1H), 6.38 (d, 1H), 6.80–7.25 (m, 5H), 7.27–7.40 (m, 5H). MS (TOF) m/z = 775.008 $[M+Na]^+$, 791.11 $[M+K]^+$. Analytical HPLC: t_R = 33.71 min (system A), 28.44 min (system B).

5.1.3.46. (*R*)-*N*-(3-Methyl)butyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Dmt-NH$^{i\ Pe,\ 5f}$). Compound **5f** was prepared from 3-methylbutylamine and Boc-Dmt-OH by general methods A and B. Yield: 99%. ^{1}H NMR ($CDCl_3$) δ = 0.90 (d, 2× 3H), 1.30–1.42 (m, 2H+3H), 1.55–1.67 (m, 1H+3H), 3.20–3.45 (m, 2H), 4.20 (s, 1H), 4.46 (s, 2H), 4.3–4.6 (br s, 1H), 6.85–7.05 (m, 1H). MS (LC) m/z = 230.89 $[M+H]^+$. Analytical HPLC: t_R = 18.44 min (system A).

5.1.3.47. (*R*)-*N*-(3-Methyl)butyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenyl]butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Apns-Dmt-NH^{i}Pe, 6f). Compound **6f** was prepared from Boc-Apns-OH and compound **5f** by general methods A and B. Yield: 50%. ^{1}H NMR ($CDCl_3$) δ = 0.70–0.88 (m, 2× 3H), 1.30–1.50 (m, 1H+2H+2× 3H), 2.78–3.10 (m, 2H), 3.12–3.42 (m, 2H), 3.70–3.85 (m, 1H), 4.26–4.48 (m, 1H+2H), 4.92–5.07 (m, 1H), 7.06–7.14 (m, 2H), 7.21–7.40 (m, 5H), 7.9–8.1 (br s, 1H). OH not observed. MS (TOF) m/z = 407.959 $[M+H]^+$, 430.041 $[M+Na]^+$, 445.92 $[M+K]^+$. Analytical HPLC: t_R = 22.44 min (system A).

5.1.3.48. (*R*)-*N*-(3-Methyl)butyl-3-{(2*S*,3*S*)-3-[(2*S*)-2-amino-3,3-dimethyl]butanoylamino-2-hydroxy-4-phenyl}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Tle-Apns-Dmt-NH^{i}Pe, 7f). Compound **7f** was prepared from Boc-Tle-OH and compound**6f** by general methods A and B. Yield: 66%. ^{1}H NMR ($CDCl_3$) δ = 0.68–1.00 (m, 5× 3H), 1.30–1.60 (m, 1H+2H+2× 3H), 2.77–3.85 (m, 2× 1H+2× 2H), 4.20–5.20 (m, 2× 1H+2H), 6.5–6.7 (br s, 1H), 7.03–7.27 (m, 5H), 8.0–8.2 (br s, 1H). OH and NH_2 not observed. MS (TOF) m/z = 521.049 $[M+H]^+$, 543.142 $[M+Na]^+$, 559.076 $[M+K]^+$. Analytical HPLC: t_R = 23.55 min (system A).

5.1.3.49. (*R*)-*N*-(3-Methyl)butyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-amino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (H-Phg-Tle-Apns-Dmt-NH^{i}Pe, 8af). Compound **8af** was prepared from Boc-Phg-OH and compound **7f** by general methods A and B. Yield: 65%. ^{1}H NMR ($CDCl_3$) δ = 0.60–0.90 (m, 5× 3H), 1.25–1.63 (m, 1H+2H+2× 3H), 2.54–3.50 (m, 2× 2H), 3.80–5.32 (m, 5× 1H+2H), 6.35–6.45 (m, 1H), 6.60–6.70 (m, 1H), 6.75–6.85 (m, 1H), 6.90–7.00 (m, 1H), 7.07–7.26 (m, 5H), 7.28–7.60 (m, 5H). OH and NH_2 not observed. MS (TOF) m/z = 653.911 $[M+H]^+$, 676.319 $[M+Na]^+$, 692.083 $[M+K]^+$. Analytical HPLC: t_R = 25.24 min (system A), 13.94 min (system B).

5.1.3.50. (*R*)-*N*-(3-Methyl)butyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-butyrylamino-2-phenyl]acetylamino-3,3-dimethyl}butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{n}PrCO-Phg-Tle-Apns-Dmt-NH^{i}Pe, 8df). Compound **8df** was prepared from *n*-butyric acid and compound**8af** by general method A. Yield: 50%. ^{1}H NMR ($CDCl_3$) δ = 0.75–0.99 (m, 6× 3H), 1.30–1.73 (m, 1H+2× 2H+2× 3H), 2.25 (t, 2H), 2.60–2.85 (m, 2H), 3.10–3.42 (m, 2H), 3.95–5.00 (m, 4× 1H+2H), 5.44–5.54 (m, 1H), 6.10 (d, 1H), 6.18 (d, 1H), 6.56 (t, 1H), 6.72 (d, 1H), 6.90–7.26 (m, 5H), 7.27–7.42 (m, 5H). OH not observed. MS (TOF) m/z = 724.845 $[M+H]^+$, 746.461 $[M+Na]^+$, 762.532 $[M+K]^+$. Analytical HPLC: t_R = 31.79 min (system A), 24.22 min (system B).

5.1.3.51. (*R*)-*N*-(3-Methyl)butyl-3-{{(2*S*,3*S*)-3-{(2*S*)-2-[(2*S*)-2-(3-methylbutanoyl)amino-2-phenyl]acetylamino-3,3-dimethyl}-butanoylamino-2-hydroxy-4-phenyl}}butanoyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (^{i}BuCO-Phg-Tle-Apns-Dmt-NH^{i}Pe, 8hf). Compound **8hf** was prepared from isovaleric acid and compound **8af** by general method A. Yield: 51%. ^{1}H NMR ($CDCl_3$) δ = 0.73–0.98 (m, 7× 3H), 1.30–1.60 (m, 1H+2H+2× 3H), 2.07–2.20 (m, 1H+2H), 2.58–2.85 (m, 2H), 3.07–3.42 (m, 2H), 4.00–5.00 (m, 4× 1H +2H), 5.53 (t, 1H), 6.14–6.29 (m, 1H), 6.30–6.40 (br s, 1H), 6.60 (t, 1H), 6.75 (d, 1H), 6.90–7.26 (m, 5H), 7.27–7.40 (m, 5H). OH not observed. MS (TOF) m/z = 738.641 $[M+H]^+$, 760.501 $[M+Na]^+$, 776.499 $[M+K]^+$. Analytical HPLC: t_R = 32.75 min (system A), 25.83 min (system B).

5.2. HIV-1 and HTLV-I protease inhibition assays

Compounds **8aa**–**8id** were evaluated for HIV-1 protease inhibitory activity at 50 nM of the test compound, and HTLV-I protease inhibitory activity as IC_{50} values, using previously reported procedures.[21] The HTLV-I protease used in the assay was an L40I mutant of the wild-type protease to prevent autolysis and improve stability.[23]

5.3. Quantitative structure–activity relationship study

QSAR equations were derived using Microsoft Excel and the 'least squares' method to calculate a straight line that best fits the data. Scores were assigned as described in Section 3.

5.4. Computer-assisted docking experiments

Computer-assisted docking experiments of an HTLV-I protease inhibitor with an HTLV-I protease L40I mutant were performed as previously described,[22] except that the target inhibitor is compound **8dc**.

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