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Design, Synthesis, and X-ray Crystallographic Analysis of a Novel Class of HIV-1 Protease Inhibitors†

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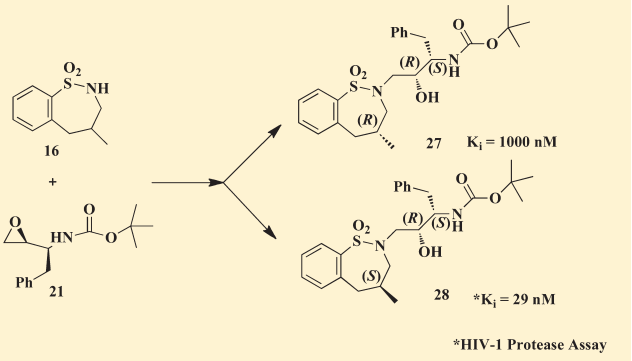
ABSTRACT: In the present paper, design, synthesis, X-ray crystallographic analysis, and HIV-1 protease inhibitory activities of a novel class of compounds are disclosed. Compounds **28**−**30**, **32**, **35**, and **40** were synthesized and found to be inhibitors of the HIV-1 protease. The crucial step in their synthesis involved an unusual endo radical cyclization process. Absolute stereochemistry of the three asymmetric centers in the above compounds have been established to be (4*S*,2′*R*,3′*S*) for optimal potency. X-ray crystallographic analysis has been used to determine the binding mode of the inhibitors to the HIV-1 protease.



INTRODUCTION

In a recent publication[1] we have described the synthesis of conformationally restricted sulfonamides represented by formula **1** wherein the R′ substituents are alkyl, aryl, and arylalkyl groups (Figure 1). The ring size could be six, seven, or eight atoms. Our expectation is that these cyclic sulfonamides will assume fewer conformations compared to open chain sulfonamides and thus serve as valuable pharmacophores in drug discovery. In this communication we disclose the design and synthesis of compounds, represented by the general structure **2**, that have been found to be inhibitors of HIV-1 protease. Several drugs such as **3**, **4**, and **5**, which are HIV-1 protease inhibitors,[2−5] are widely and successfully used in AIDS patients. In general our compounds represent novel pharmacophores and are less potent inhibitors of HIV-1 protease, when compared with the compounds mentioned above. Our hope is that with further changes in structures **2**, these compounds might show improvements in the spectrum of activity against resistant organisms and also show improvements in pharmacokinetic properties.

RESULTS AND DISCUSSION

Compounds represented by structure **1** were synthesized using a radical cyclization process. Thus, compound **6**, when treated with tributyltin hydride (TBTH) in refluxing toluene solution and in the presence of AIBN, yielded **1** via **7** and **8** (Scheme 1).

Using similar approach, we synthesized compounds **15**−**20** from **9**−**14**, respectively (Scheme 2). This allowed us to synthesize compounds represented by structure **2** by derivitization of the free −NH function.

Thus, treatment of **15** with (2*S*,3*S*)-1,2-epoxy-3- (bocamino)-4-phenylbutane (**21**) in DMF solution and in the presence of cesium carbonate yielded **22** (Scheme 3). Compound **22**, when treated with 30% trifluoroacetic acid in DCM, yielded intermediate **23a**. The crude intermediate **23a** was further reacted with isopropyl 1*H*-imidazole-1-carboxylate, which was generated by reacting carbonyldiimidazole with isopropanol, in the presence of *N*,*N*′-diisopropylethylamine to yield compound **23**. Compound **22**, when tested in HIV-protease assay, showed a K_i of 470 nM, and **23** was inactive. As expected, these results suggest the importance of the free hydroxyl group in **22** for HIV-1 protease inhibitory activity.

Furthermore, we decided to study the effect of substitution on the cyclic sulfonamide moiety in both the aromatic and alicyclic portion of **22**, as well as study the nature of substituted carbamates and amides required for optimizing antiviral potency. The results of these studies are summarized below.

Treatment of **16** with **21** in DMF solution and in the presence of cesium carbonate yielded a mixture of diastereoisomers **24**,

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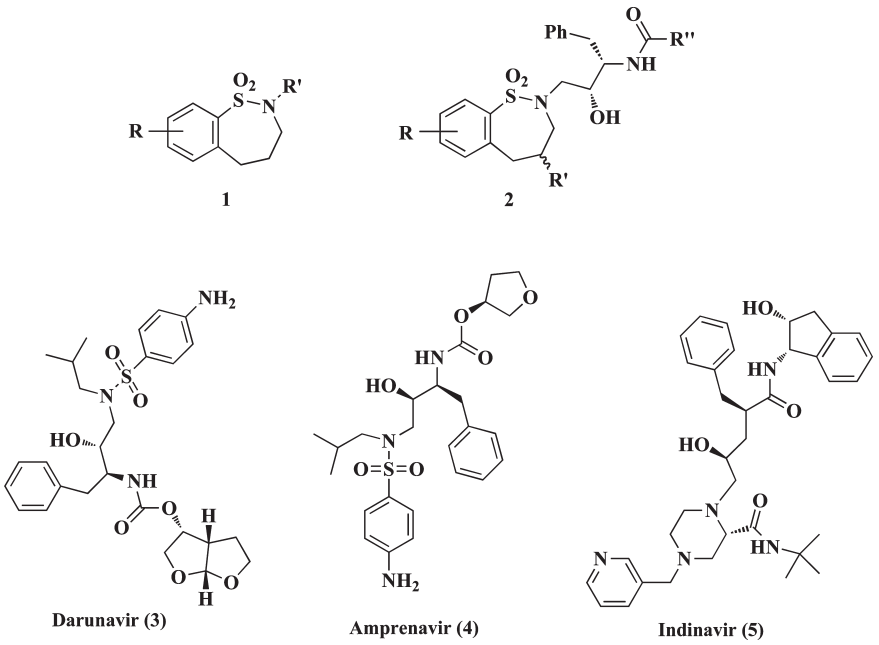
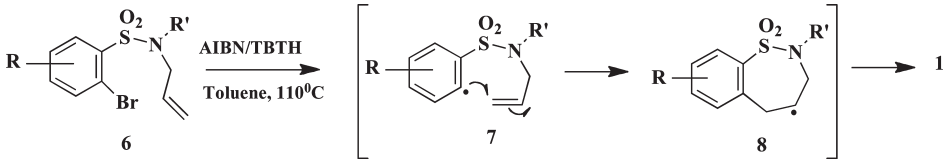
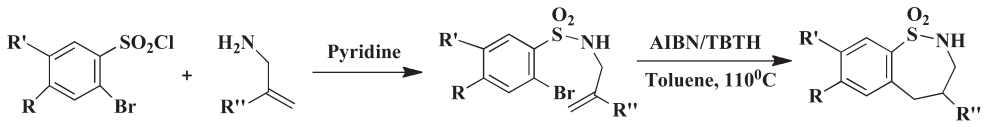


Figure 1. HIV-1 protease inhibitors.

Scheme 1. Synthesis of Seven-Membered Cyclic Sulfonamide Using Radical Cyclization



Scheme 2. Synthesis of Conformationally Restricted Sulfonamides 15−20



9 R=R'= H; R''= H
10 R=R'= H; R''= CH_3
11 R= F; R'= H; R''= CH_3
12 R= CF_3; R'= H; R''= CH_3
13 R= H; R'= CF_3; R''= CH_3
14 R= OCH_3; R'= H; R''= CH_3

15 R=R'= H; R''= H
16 R=R'= H; R''= CH_3
17 R= F; R'= H; R''= CH_3
18 R= CF_3; R'= H; R''= CH_3
19 R= H; R'= CF_3; R''= CH_3
20 R= OCH_3; R'= H; R''= CH_3

which could not be separated. However, on treatment with trifluoroacetic acid in dichloromethane solution, **24** yielded **25** and **26**, which could be separated using preparative TLC (Scheme 4). X-ray crystallographic analysis of **25** (Figure 2) established its absolute stereochemistry to be (4*R*,2′*R*,3′*S*), and therefore, the absolute stereochemistry of **26** is (4*S*,2′*R*,3′*S*).

Treatment of **25** in THF solution with di-*tert*-butyl dicarbonate and diisopropylethylamine yielded **27**, and similarly **26** yielded **28**. In HIV protease assay, **28** with K_i of 29 nM, was considerably more potent than **22** and it was also more potent than the diastereoisomer **27** which showed a K_i of 1000 nM. These results demonstrated the importance of the methyl substituent at C4 and also the absolute stereochemistry at C4 to be (*S*) for optimum HIV protease inhibitory activity. Thus, **28** with (4*S*,2′*R*,3′*S*) geometry was considerably more potent than **27** with (4*R*,2′*R*,3′*S*) geometry.

Using the above procedure for the synthesis of **28**, we prepared a number of carbamates maintaining (4*S*,2′*R*,3′*S*) geometry and then tested them in the HIV-1 protease inhibitor assay. The results are summarized in Table 1. We prepared the corresponding (4*R*,2′*R*,3′*S*) diastereoisomers and found them to be considerably less active (results not shown).

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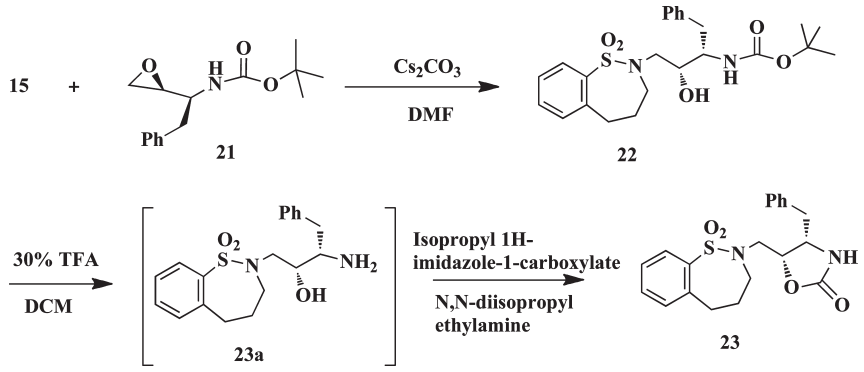
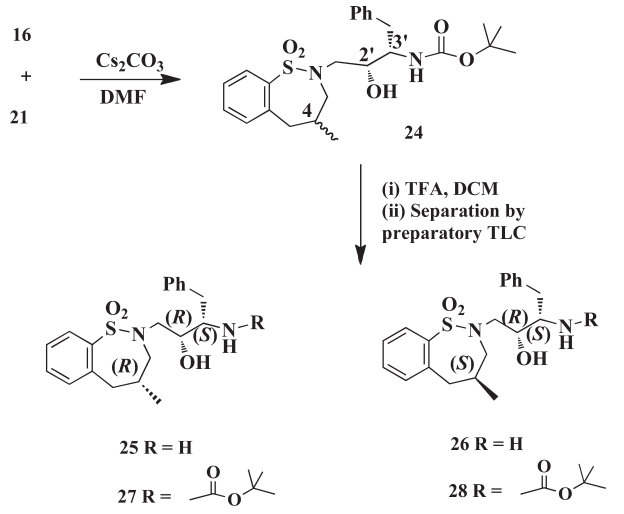
Scheme 3. Synthesis of Compounds 22 and 23



Scheme 4. Synthesis of Diastereoisomers 27 and 28



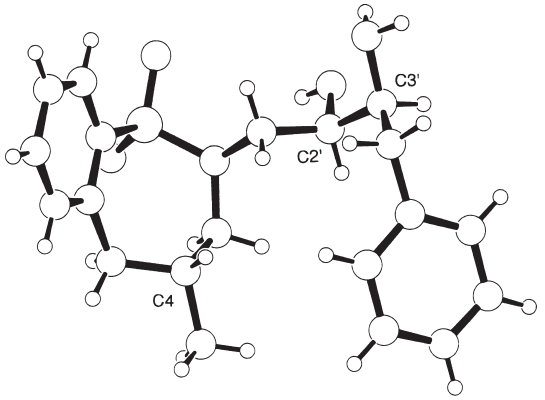
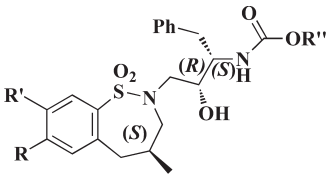


Figure 2. X-ray crystallographic structure of compound **25**.

Table 1. Novel HIV Protease Inhibitors



compd	substitution	K_i (nM)
29	R = R′ = H; R″ = allyl	27
30	R = R′ = H; R″ = Et	34
31	R = R′ = H; R″ = Ph	350
32	R = F; R′ = H; R″ = *tert*-butyl	20
33	R = CF_3; R′ = H; R″ = *tert*-butyl	186
34	R = H; R′ = CF_3; R″ = *tert*-butyl	100
35	R = OCH_3; R′ = H; R″ = *tert*-butyl	19

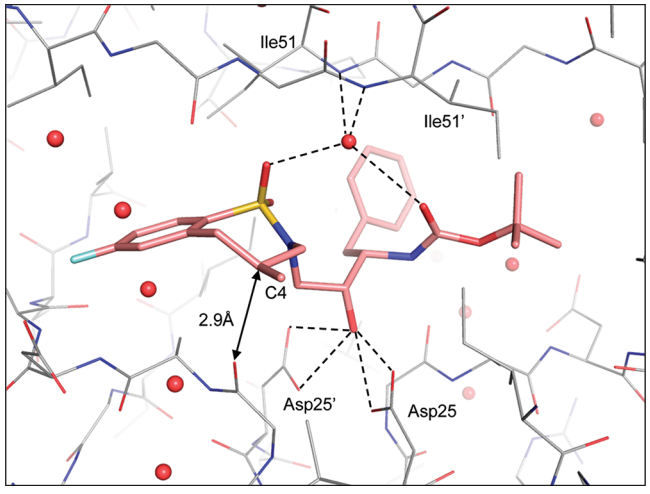


Figure 3. X-ray crystallographic structure of **32** bound to HIV-1 protease.

The X-ray crystallographic structure of compound **32** was solved bound to the HIV-1 protease. Compound **32** occupies the active site cavity and makes hydrogen bonds to the catalytic aspartic acid residues Asp25 and Asp25′ through the hydroxyl group (Figure 3). The sulfonamide and the carbamate carbonyl form hydrogen bonds with a structural water molecule, which in turn hydrogen-bonds with Ile50 and Ile50′ of the protease.

In addition to these favorable hydrogen bonding stabilization effects, the C4-Me group of compound **32** has extensive hydrophobic interactions with side chains of Leu23, Val82, and Ile84.

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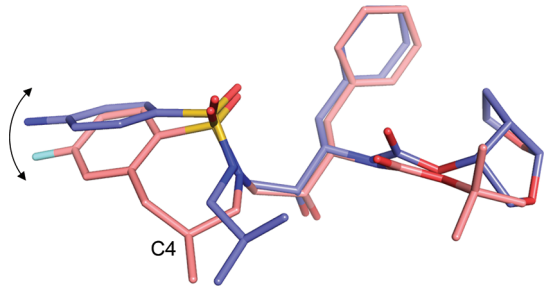
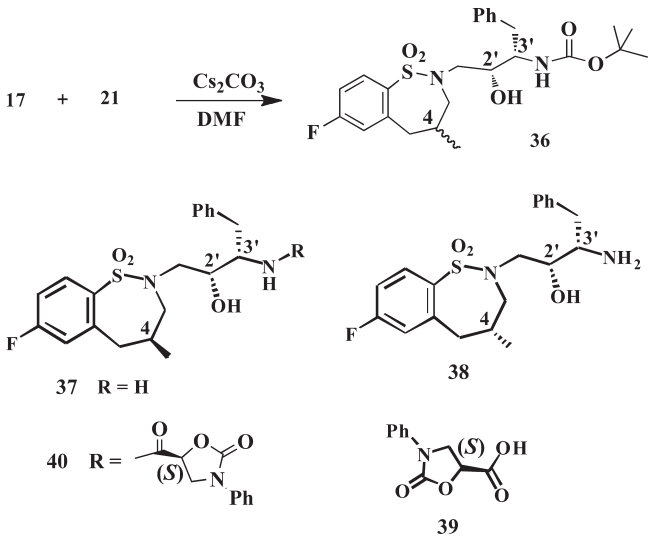


Figure 4. Overlay of **32** with darunavir (PDB code 2hs1). Rotation of cyclized fluorophenyl with respect to noncyclized aromatic plane is shown with arrow.

Scheme 5. Synthesis of Amide Inhibitor 40



Furthermore, C4-Me (*S* configuration) in compound **32** is 2.9 Å away from the carbonyl oxygen of Gly27′. If the C4-Me configuration is changed to (*R*) instead of (*S*), an unfavorable repulsion would be created between the C4-Me and the Gly27′ carbonyl group and the interactions to the hydrophobic pocket would be lost. These results support the experimental observation that compounds with the C4 methyl group in the (*S*) configuration possess optimum activity.

When superimposing the X-ray structure of **32** with darunavir (**3**), a similar acyclic inhibitor, there is a nice overlay with the exception of the aromatic sulfonamide plane, which is rotated by 42° (Figure 4). The cyclizing linker, including C4, bridges the hydrophobic area of the active site occupied by the isobutyl of darunavir (**3**).

To investigate whether the carbamates in this series of compounds could be replaced by amides, we treated compound **17** with **21** and obtained a mixture of diastereoisomers **36** that again could not be separated (Scheme 5). Compound **36** on treatment with trifluoro acetic acid yielded **37** and **38**, which were separated using preparative TLC. Diastereoisomer **37** with (4*S*,2′*R*,3′*S*) geometry was treated with **39**[6] in dichloromethane solution and dicyclohexyl carbodiimide to yield **40** which when tested in HIV-1 protease assay had a K_i of 30 nM.

CONCLUSION

On the basis of a novel pharmacophore structure containing a conformationally restricted sulfonamide, a class of HIV-1 protease inhibitors was designed and synthesized. An unusual endo radical cyclization process was used as a key step in their synthesis. Several analogues were synthesized to establish a preliminary structure−activity relationship, and the importance of the absolute stereochemistry of the three asymmetric centers for optimum activity was established. These compounds were shown to possess HIV-1 protease inhibitory activity. X-ray crystallographic analysis of compound **32**, when bound to the HIV-1 protease, demonstrated that it binds to a similar pocket as known inhibitors, such as darunavir. The 2.9 Å distance and the geometry between C4-Me and the carbonyl oxygen of Gly27′ explain the strong preference for the (*S*) stereochemistry of the C4 position.

Further work is in progress to improve the potency based on the X-ray results described above. We are exploring the possibility of optimizing the C4-methyl binding site and also incorporating different substitutions on the aromatic ring of the cyclic sulfonamide moiety. Modification of the carbamate functionality to capture other interactions with the protein backbone also will be explored. These cyclic sulfonamide inhibitors offer an exciting opportunity to expand the scope of compounds active against HIV-1 protease.

EXPERIMENTAL SECTION

General Procedures. All the reactions were performed under a nitrogen atmosphere with magnetic stirring. Air- and moisture-sensitive reagents were transferred via disposable syringes. Anhydrous solvents and commercially available chemicals were used without further drying. TLC was performed on Analtech silica gel glass plates (250 μm) and visualized with a UV lamp. NMR spectra were recorded with Varian 400 MHz spectrometer using $CDCl_3$ as solvent unless otherwise noted. The purity of the compounds was established using various chromatographic techniques including HPLC and was judged to be >95%.

General Procedure for Sulfonamide Formation, 9−14. To a solution of allylamine/2-methylallylamine (1.1 equiv) in pyridine was added 2-bromobenzenesulfonyl chloride (1 equiv) dissolved in pyridine. The mixture was stirred at room temperature for 8−10 h under nitrogen. TLC was used to monitor the progress of the reaction. After the reaction was complete, the reaction mixture was neutralized with 30% HCl and then the aqueous portion was extracted with dichloromethane (DCM). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness to yield the crude compound which was purified by column chromatography using 15% ethyl acetate in hexane to yield the pure compound.

***N*-Allyl-2-bromobenzenesulfonamide (9).** The title compound was obtained from 2-bromobenzenesulfonyl chloride and allylamine following the general procedure for sulfonamide formation. Crystals were obtained from DCM and hexane (1:4). Yield: 70%. Mp 68−70 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.14 (dd, J = 7.69, 1.88 Hz, 1H), 7.74 (dd, J = 7.74, 1.36 Hz, 1H), 7.45−7.40 (m, 2H), 5.72−5.62 (m, 1H), 5.25−5.05 (m, 3H), 3.57 (t, J = 6.11 Hz, 2H).

2-Bromo-*N*-(2-methylallyl)benzenesulfonamide (10). The title compound was prepared following the general procedure for sulfonamide formation by using 2-bromobenzenesulfonyl chloride and 2-methylallylamine. Crystals were obtained from DCM and hexane (1:4). Yield: 53%. Mp 83−85 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.15 (dd, J = 7.73, 1.85 Hz, 1H), 7.76 (dd, J = 7.72, 1.40 Hz, 1H), 7.45−7.40 (m, 2H), 5.23 (b, 1H), 4.91 (s, 1H), 4.85 (s, 1H), 3.47 (d, J = 6.42 Hz, 2H), 1.71 (s, 3H).

2-Bromo-4-fluoro-*N*-(2-methylallyl)benzenesulfonamide (11). The title compound was prepared following the general procedure for sulfonamide formation by using 2-bromo-4-fluorobenzenesulfonyl chloride and 2-methylallylamine. Crystals were obtained from DCM and hexane (1:4). Yield: 83%. Mp 51−53 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.15 (dd, J = 8.84, 5.80 Hz, 1H), 7.48 (dd, J = 7.90, 2.51 Hz, 1H), 7.19−7.16 (m, 1H), 5.16 (b, 1H), 4.89 (s, 1H), 4.84 (s, 1H), 3.46 (d, J = 6.37 Hz, 2H), 1.70 (s, 3H). MS (ESI): m/z 307.94 $[M + H]^+$

2-Bromo-*N*-(2-methylallyl)-4-(trifluoromethyl)benzenesulfonamide (12). The title compound was prepared following the general procedure for sulfonamide formation, from 2-bromo-4-trifluoromethylbenzenesulfonyl chloride and 2-methylallylamine. Crystals were obtained from DCM and hexane (1:5). Yield: 72%. Mp 102−103 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.26 (d, J = 8.23 Hz, 1H), 8.01 (s, 1H), 7.74 (d, J = 8.23 Hz, 1H), 5.2 (t, J = 6.4, 1H), 4.88 (dd, J = 18.27, 0.71 Hz, 2H), 3.51 (d, J = 6.35 Hz, 2H), 1.71 (s, 3H). MS (FAB): m/z 358.1 $[M + H]^+$.

2-Bromo-*N*-(2-methylallyl)-5-(trifluoromethyl)benzenesulfonamide (13). The title compound was prepared following the general procedure for sulfonamide formation, from 2-bromo-5-trifluoromethylbenzenesulfonyl chloride and 2-methylallylamine. Crystals were obtained from DCM and hexane (1:5). Yield: 71%. Mp 85−86 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.38 (s, 1H), 7.89 (d, J = 8.32 Hz, 1H), 7.66 (d, J = 8.23 Hz, 1H), 5.22 (t, J = 5.90 Hz, 1H), 4.87 (d, J = 21.55 Hz, 2H), 3.52 (d, J = 6.36 Hz, 2H), 1.69 (s, 3H). MS (FAB): m/z 358.1 $[M + H]^+$.

2-Bromo-4-methoxy-*N*-(2-methylallyl)benzenesulfonamide (14). The title compound was prepared following the general 555procedure for sulfonamide formation, from 2-bromo-4-methoxybenzenesulfonyl chloride and 2-methylallylamine. Crystals were obtained from DCM and hexane (1:4). Yield: 31%. Mp 69−71 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.01 (d, J = 8.84 Hz, 1H), 7.22 (d, J = 2.31 Hz, 1H), 6.90 (dd, J = 8.81, 2.28 Hz, 1H), 5.13 (t, J = 5.99 Hz, 1H), 4.84 (d, J = 26.57 Hz, 2H), 3.84 (s, 3H), 3.39 (d, J = 6.34 Hz, 2H), 1.68 (s, 3H).

General Procedure for Radical Reaction, 15−20. Compound **9** (1 equiv) was dissolved in toluene, and to the mixture was added azobisisobutyronitrile (AIBN) (0.2 equiv). The above solution was heated to about 60−70 °C, and then tributyltin hydride (TBTH) (1.1 equiv) was added slowly under nitrogen. After the addition was complete, the reaction mixture was refluxed for 4−6 h. Upon completion (followed by TLC), the reaction mixture was evaporated to dryness. The residue was extracted with DCM and washed with water. The organic layers were combined, dried over sodium sulfate, and concentrated to yield the crude compound which was further purified by column chromatography using ethyl acetate and hexane to yield the pure compound.

2,3,4,5-Tetrahydro-1*H*-1λ^6,2-benzothiazepine-1,1-dione (15). The title compound was prepared starting from **9** following the general procedure for radical reaction, giving a white solid. Crystals were obtained from DCM and hexane (1:4). Yield: 44%. Mp 107−108 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.88 (d, J = 7.69 Hz, 1H), 7.41 (dt, J = 7.49, 1.30 Hz, 1H), 7.35−7.22 (m, 2H), 4.58 (b, 1H), 3.60 (dd, J = 10.71, 6.11 Hz, 2H), 3.30−3.25 (m, 2H), 1.80−1.79 (m, 2H).

4-Methyl-2,3,4,5-tetrahydro-1*H*-1λ^6,2-benzothiazepine-1,1-dione (16). The title compound was prepared starting from **10** following the general procedure for radical reaction, giving a white solid. Crystals were obtained from DCM and hexane (1:5). Yield: 85%. Mp 121−123 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.85 (dd, J = 7.76, 1.23 Hz, 1H), 7.41 (dt, J = 7.53, 7.53, 1.32 Hz, 1H), 7.28−7.21 (m, 2H), 4.70 (b, 1H), 3.6−3.0 (m, 4H), 1.93 (b, 1H), 0.93 (d, J = 6.87 Hz, 3H).

7-Fluoro-4-methyl-2,3,4,5-tetrahydro-1*H*-1λ^6,2-benzothiazepine-1,1-dione (17). The title compound was prepared from compound **11** following the general procedure for radical reaction, giving a white solid. Crystals were obtained from DCM and hexane (1:5). Yield: 86%. Mp 144−146 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.86 (dd, J = 9.14, 5.70 Hz,1H), 6.99−6.91 (m, 2H), 4.70 (t, J = 6.23 Hz, 1H), 3.6−3.0 (m, 4H), 1.94 (b, 1H), 0.94 (d, J = 6.84 Hz, 3H). MS: m/z 230.05 $[M + H]^+$.

4-Methyl-7-(trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-1λ^6,2-benzothiazepine-1,1-dione (18). The title compound was prepared from compound **12** following the general procedure for radical reaction, giving a white solid. Crystals were obtained from DCM and hexane (1:4). Yield: 71%. Mp 154−155 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.03 (d, J = 8.12 Hz,1H), 7.59 (d, J = 8.12 Hz, 1H), 7.52 (s, 1H), 4.61 (t, J = 5.89 Hz, 1H), 3.61−3.15 (m, 4H), 1.97 (b, 1H), 0.97 (d, J = 6.46 Hz, 3H). MS: m/z 280.06 $[M + H]^+$.

4-Methyl-8-(trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-1λ^6,2-benzothiazepine-1,1-dione (19). The title compound was prepared from compound **13** following the general procedure for radical reaction, giving a white solid. Crystals were obtained from DCM and hexane (1:4). Yield: 73%. Mp 99−101 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.15 (s,1H), 7.67 (d, J = 7.74 Hz,1H), 7.39 (d, J = 7.89 Hz,1H), 4.76 (b, 1H), 3.60−3.10 (m, 4H), 1.97 (b, 1H), 0.96 (d, J = 6.73 Hz, 3H). MS: m/z 280.06 $[M + H]^+$.

7-Methoxy-4-methyl-2,3,4,5-tetrahydro-1*H*-1λ^6,2-benzothiazepine-1,1-dione (20). The title compound was prepared from compound **14** following the general procedure for radical reaction, giving an oil. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.71 (d, J = 8.42 Hz, 1H), 6.66−6.1 (m, 2H), 4.56 (t, J = 6.78 Hz, 1H), 3.76 (s, 3H), 3.50−2.90 (m, 4H), 1.85 (b, 1H), 0.86 (d, J = 7.32 Hz, 3H). HRMS calcd for $C_{11}H_{16}NO_3S$ $[M + H]^+$ 242.0845; found 242.0852.

***tert*-Butyl *N*-[(1*S*,2*R*)-1-Benzyl-3-(1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl)-2-hydroxypropyl]carbamate (22).** To a solution of compound **15** (75 mg, 0.37 mmol) in *N*,*N*-dimethylformamide (DMF) (3 mL) was added (2*S*,3*S*)-1,2-epoxy-3-(bocamino)-4-phenylbutane (**21**) (100 mg, 0.37 mmol), followed by the addition of cesium carbonate (247 mg, 0.75 mmol). The reaction mixture was stirred for 10−12 h at room temperature under nitrogen. TLC was used to monitor the progress of the reaction. The reaction mixture was filtered, diluted with ethyl acetate, and then washed with water. The combined organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to yield the crude compound which was purified by column chromatography using 25% ethyl acetate in hexane to yield the title compound. Yield: 57%. HPLC: >98% pure. NMR data were consistent with the proposed structure. MS (ESI): m/z 405.05 $[M + H]^+$.

2-{[(4*S*,5*R*)-4-Benzyl-2-oxo-1,3-oxazolidin-5-yl]methyl}-2,3,4,5-tetrahydro-1*H*-1 λ^6,2-benzothiazepine-1,1-dione (23). To a solution of compound **22** (50 mg, 0.13 mmol) in DCM was added 30% trifluoroacetic acid in DCM solution, and the mixture was stirred for 1 h to obtain compound **23a**. In flask 2, isopropyl alcohol (1 equiv) and carbonyl diimidazole (1 equiv) were allowed to react. Compound **23a** was dissolved in toluene, and the mixture was maintained at 0 °C. To this was added *N*,*N*-diisopropylethylamine followed by the mixture in flask 2. The resulting reaction mixture was stirred at room temperature for 6 h. The reaction mixture was evaporated to dryness and was purified by column chromatography using 20% ethyl acetate in hexane as mobile phase. Yield: 31%. HPLC: >98% pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.90 (dd, J = 7.75, 1.11 Hz, 1H), 7.47 (dt, J = 7.45, 1.14 Hz, 1H), 7.39−7.22 (m, 5H), 7.11 (d, J = 6.91 Hz, 2H), 4.95−5.01 (m, 1H), 4.79 (s, 1H), 4.19−4.05 (m, 2H), 3.79−3.42 (m, 2H), 3.38 (dd, J = 14.86, 3.22 Hz, 1H), 3.12−2.92 (m, 2H), 2.87 (dd, J = 13.29, 3.30 Hz, 1H), 2.52 (dd, J = 12.83, 11.61 Hz, 1H), 1.90−1.79 (m, 2H).

***tert*-Butyl *N*-[(1*R*,2*S*)-1-Benzyl-2-hydroxy-3-(4-methyl-1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl)propyl]carbamate (24).** To a solution of compound **16** (187 mg, 0.71 mmol) in DMF (3 mL) was added compound **21** (150 mg, 0.71 mmol) followed by cesium carbonate (462 mg, 1.42 mmol). The mixture was stirred for 10−12 h at room temperature under nitrogen. TLC was used to monitor the progress of the reaction. The reaction mixture was

filtered. Water was added and then extracted using ethyl acetate. The combined organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated yielding the crude compound which was purified by column chromatography using 25% ethyl acetate in hexane to yield compound **24** (170 mg, 50%) as an oil. HPLC: >98% pure. ^{1}H NMR data were consistent with the structure (mixture of diastereoisomers). MS (ESI): m/z 457.05 $[M - 18 + H]^+$.

(4*R*)-2-[(2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutyl]-4-methyl-2,3,4,5-tetrahydro-1*H*-1λ^6,2-benzothiazepine-1,1-dione (25) and (4*S*)-2-[(2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutyl]-4-methyl-2,3,4,5-tetrahydro-1*H*-1λ^6,2-benzothiazepine-1,1-dione (26). Compound **24** (125 mg, 0.263 mmol) was dissolved in a mixture of DCM and trifluoroacetic acid (1.5 mL/1.5 mL), and the mixture was stirred at room temperature under nitrogen for 3−4 h. TLC was used to monitor the progress of the reaction. After the reaction was complete, the reaction mixture was basified with sodium hydroxide solution (50%) and the aqueous portion was extracted with ethyl acetate. The combined organic layer was dried with anhydrous sodium sulfate and evaporated to yield the crude compound. Compounds **25** (40 mg, 41%) and **26** (50 mg, 51%) were separated by preparative TLC (6% methanol in chloroform) and were recrystallized from DCM/hexane (1:4).

Compound ***25***. Mp 153−155 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.91 (dd, J = 7.74, 1.24 Hz, 1H), 7.48−7.41 (m, 1H), 7.36−7.14 (m, 7H), 3.87 (t, J = 13.48 Hz, 1H), 3.60 (s, 2H), 3.19−3.03 (m, 3H), 3.00−2.86 (m, 2H), 2.72 (s, 1H), 2.54 (dd, J = 13.51, 9.38 Hz, 1H), 1.99 (s, 4H), 1.02 (d, J = 6.81 Hz, 3H). HRMS calcd for $C_{20}H_{27}N_2O_3S$ $[M + H]^+$ 375.1737; found 375.1737.

Compound ***26***. Mp 117−119 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.92 (dd, J = 7.69, 1.21 Hz, 1H), 7.45 (dt, J = 7.54, 7.53, 1.34 Hz, 1H), 7.36−7.17 (m, 7H), 3.86 (ddd, J = 16.10, 11.48, 7.92 Hz, 2H), 3.57 (s, 1H), 3.35 (s, 1H), 3.21−3.06 (m, 2H), 2.98 (dd, J = 13.29, 3.12 Hz, 1H), 2.90−2.62 (m, 2H), 2.44 (dd, J = 13.45, 10.22 Hz, 1H), 2.04−1.95 (m, 3H), 1.28−1.20 (m, 1H), 1.02 (d, J = 6.80 Hz, 3H). HRMS calcd for $C_{20}H_{27}N_2O_3S$ $[M + H]^+$ 375.1737; found 375.1736.

General Procedure for Carbamate Formation, 27, 28, 34−40. To an ice cold solution of compound **25** (1 equiv) in THF, diisopropylethylamine (DIPEA) (1 equiv) was added followed by the addition of di-*tert*-butyl dicarbonate/alkyl chlorofomate (1 equiv) in THF. The reaction mixture was slowly allowed to reach room temperature and stirring was continued for 10−12 h under nitrogen. The progress of the reaction was monitored by TLC. When the reaction was complete, the reaction mixture was diluted with water and extracted with chloroform. The combined organic layer was dried over anhydrous sodium sulfate and evaporated to yield the crude compound. The crude compound was purified by column chromatography using ethyl acetate and hexane.

***tert*-Butyl *N*-{(1*R*,2*S*)-1-Benzyl-2-hydroxy-3-[(4*R*)-4-methyl-1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl]propyl}carbamate (27).** The title compound was synthesized from compound **25**, diisopropylethylamine (DIPEA), and di-*tert*-butyl dicarbonate using the general procedure for carbamate formation. Yield: 89%. HPLC: >98% pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.89 (dd, J = 7.59, 1.09 Hz, 1H), 7.44 (dt, J = 7.53, 7.50, 1.39 Hz, 1H), 7.33 (dt, J = 7.64, 7.55, 1.09 Hz, 1H), 7.28−7.14 (m, 6H), 4.71 (b, 1H), 3.94−3.40 (m, 4H), 3.24−2.59 (m, 5H), 1.88 (s, 1H), 1.60 (s, 2H), 1.32 (s, 9H), 0.99 (d, J = 6.73 Hz, 3H). HRMS calcd for $C_{25}H_{35}N_2O_5S$ $[M + H]^+$ 475.2261; found 475.2271.

***tert*-Butyl *N*-{(1*R*,2*S*)-1-Benzyl-2-hydroxy-3-[(4*S*)-4-methyl-1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl]propyl}carbamate (28).** The title compound was synthesized from **26**, DIPEA, and di-*tert*-butyl dicarbonate using the general procedure for carbamate formation. Yield: 75%. HPLC: >98% pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.91 (dd, J = 7.69, 1.32 Hz, 1H), 7.43 (dt, J = 7.48, 7.46, 1.37 Hz, 1H), 7.36−7.17 (m, 7H), 4.69 (d, J = 6.73 Hz, 1H), 4.10 (s, 1H), 3.95−3.73 (m, 3H), 3.55 (s, 1H), 3.24 (s, 1H), 3.10 (dd, J = 14.84, 5.16 Hz, 1H), 3.02−2.86 (m, 1H), 2.70 (d, J = 11.08 Hz, 2H), 1.96 (s, 1H), 1.63 (s, 1H), 1.33 (s, 9H), 0.99 (d, J = 6.70 Hz, 3H). HRMS calcd for $C_{25}H_{35}N_2O_5S$ $[M + H]^+$ 475.2261; found 475.2271.

Allyl *N*-{(1*R*,2*S*)-1-Benzyl-2-hydroxy-3-[(4*S*)-4-methyl-1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl]propyl}carbamate (29). HPLC: >98% pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.90 (dd, J = 7.74, 1.31 Hz, 1H), 7.43 (dt, J = 7.54, 7.52, 1.36 Hz, 1H), 7.37−7.16 (m, 7H), 5.81 (ddd, J = 15.76, 9.78, 5.08 Hz, 1H), 5.30−5.07 (m, 2H), 4.95 (d, J = 7.23 Hz, 1H), 4.46 (dd, J = 6.56, 3.54 Hz, 2H), 4.00−3.71 (m, 3H), 3.55 (s, 1H), 3.30−2.85 (m, 4H), 2.69 (d, J = 10.64 Hz, 2H), 1.95 (s,1H), 1.25 (s, 1H), 0.99 (d, J = 6.69 Hz, 3H). HRMS calcd for $C_{24}H_{31}N_2O_5S$ $[M + H]^+$ 459.1953; found 459.1946.

Ethyl *N*-{(1*R*,2*S*)-1-Benzyl-2-hydroxy-3-[(4*S*)-4-methyl-1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl]propyl}carbamate (30). HPLC: >98% pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.91 (dd, J = 7.70, 1.32 Hz, 1H), 7.44 (dt, J = 7.52, 7.52, 1.43 Hz, 1H), 7.35−7.16 (m, 7H), 4.89 (s, 1H), 4.05−3.77 (m, 4H), 3.55 (s, 1H), 2.90−3.21 (m, 7H), 1.96 (s, 2H), 1.2−1.15 (m, 3H), 1.00 (d, J = 6.75 Hz, 3H). HRMS calcd for $C_{23}H_{30}N_2O_5S$ $[M + Na]^+$ 469.1773; found 469.1773.

Phenyl *N*-{(1*R*,2*S*)-1-Benzyl-2-hydroxy-3-[(4*S*)-4-methyl-1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl]propyl}carbamate (31). HPLC >98% pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.93 (dd, J = 7.73, 1.31 Hz, 1H), 7.45 (dt, J = 7.50, 7.47, 1.39 Hz, 1H), 7.36−7.13 (m, 10H), 6.94 (d, J = 7.67 Hz, 2H), 5.31 (d, J = 7.68 Hz, 1H), 4.03−3.84 (m, 3H), 3.70−3.41 (m, 2H), 3.24−3.12 (m, 3H), 2.96 (dd, J = 13.50, 8.88 Hz, 1H), 2.73 (d, J = 12.33 Hz, 2H), 1.97 (s, 1H), 1.00 (d, J = 6.70 Hz, 3H). HRMS calcd for $C_{27}H_{31}N_2O_5S$ $[M + H]^+$ 495.1948; found 495.1962.

***tert*-Butyl *N*-[(1*R*,2*S*)-1-Benzyl-3-[(4*S*)-7-fluoro-4-methyl-1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl]-2-hydroxypropyl]carbamate (32).** HPLC: >98% pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.95−7.87 (m, 1H), 7.32−7.17 (m, 5H), 7.02−6.96 (m, 2H), 4.67 (d, J = 6.38 Hz, 1H), 4.08 (s, 1H), 3.95−3.71 (m, 2H), 3.54 (s, 1H), 3.24 (s, 1H), 3.16−2.83 (m, 4H), 2.69 (d, J = 10.50 Hz, 2H), 1.96 (s, 1H), 1.34 (s, 9H), 0.99 (d, J = 6.74 Hz, 3H). MS (ESI): m/z 493.21 $[M + H]^+$. HRMS calcd for $C_{20}H_{26}FN_2O_3S$ $[M + H\text{-Boc}]^+$ 393.1648; found 393.1653.

***tert*-Butyl *N*-{(1*S*,2*R*)-1-Benzyl-2-hydroxy-3-[(4*S*)-4-methyl-1,1-dioxo-7-(trifluoromethyl)-1,3,4,5-tetrahydro-2*H*-1λ^6,-2-benzothiazepin-2-yl]propyl}carbamate (33).** HPLC: >98% pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.02 (d, J = 7.98 Hz, 1H), 7.66−7.45 (m, 2H), 7.39−7.10 (m, 5H), 4.67 (d, J = 6.91 Hz, 1H), 4.18−4.02 (m, 1H), 3.99−3.67 (m, 3H), 3.72−3.48 (m, 1H), 3.43−3.19 (m, 1H), 3.09 (dd, J = 14.51, 5.32 Hz, 1H), 3.03−2.59 (m, 4H), 2.08−1.98 (m, 1H), 1.32 (s, 9H), 1.02 (d, J = 6.34 Hz, 3H). HRMS calcd for $C_{21}H_{26}F_3N_2O_3S$ $[M + H - Boc]^+$ 443.161 62; found 443.160 71.

***tert*-Butyl *N*-{(1*S*,2*R*)-1-Benzyl-2-hydroxy-3-[(4*S*)-4-methyl-1,1-dioxo-8-(trifluoromethyl)-1,3,4,5-tetrahydro-2*H*-1λ^6,-2-benzothiazepin-2-yl]propyl}carbamate (34).** HPLC: >98% pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.15 (d, J = 1.18 Hz, 1H), 7.68 (d, J = 7.95 Hz, 1H), 7.41 (d, J = 7.86 Hz, 1H), 7.31−7.19 (m, 5H), 4.69 (d, J = 7.74 Hz, 1H), 4.11 (dd, J = 14.28, 7.13 Hz, 1H), 3.97−3.18 (m, 5H), 3.16−2.71 (m, 5H), 2.03−1.84 (m, 1H), 1.32 (s, 9H), 1.01 (d, J = 6.56 Hz, 3H). HRMS calcd for $C_{26}H_{34}F_3N_2O_5S$ $[M + H]^+$ 543.2135; found 543.2144.

***tert*-Butyl *N*-{(1*S*,2*R*)-1-Benzyl-2-hydroxy-3-[(4*S*)-7-methoxy-4-methyl-1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl]propyl}carbamate (35).** HPLC: >98% pure. ^{1}H

NMR (400 MHz, $CDCl_3$) δ ppm 7.84 (d, J = 9.32 Hz, 1H), 7.32−7.15 (m, 5H), 6.77 (d, J = 5.75 Hz, 2H), 4.69 (d, J = 4.87 Hz, 1H), 4.20−3.98 (m, 1H), 3.84 (s, 3H), 3.51 (s, 1H), 3.36−2.83 (m, 5H), 2.80−2.51 (m, 3H), 2.03−1.84 (m, 2H), 1.34 (s, 9H), 0.99 (d, J = 6.71 Hz, 3H). MS (ESI): m/z 505.24 $[M + H]^+$. HRMS calcd for $C_{26}H_{36}N_2O_6S$ $[M + Na]^+$ 527.2186; found 527.2175.

(5*S*)-*N*-{(1*R*,2*S*)-1-Benzyl-3-[(4*S*)-7-fluoro-4-methyl-1,1-dioxo-1,3,4,5-tetrahydro-2*H*-1λ^6,2-benzothiazepin-2-yl]-2-hydroxypropyl}-2-oxo-3-phenyl-1,3-oxazolidine-5-carboxamide (40). To a solution of compound **37** (50 mg, 0.127 mmol) in DCM was added dicyclohexylcarbodiimide (DCCI) (24.1 mg, 0.191 mmol), and the mixture was stirred. Compound **39** (29.0 mg, 0.14 mmol) was added, and the mixture was stirred for 10−12 h at room temperature under nitrogen. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the reaction mixture was extracted with DCM and washed with water. The combined organic layer was dried with anhydrous sodium sulfate, filtered, and evaporated to yield the crude compound which was purified by preparative TLC (7.5% methanol in chloroform) (15 mg, 20%). HPLC: >98% pure. NMR data were consistent with the proposed structure. MS (ESI): m/z 582.20 $[M + H]^+$.

In Vitro HIV-1 Protease Assay. Compounds were evaluated for the ability to inhibit the enzymatic activity of HIV-1 protease in an in vitro assay.[7] Protease activity was assessed in reactions catalyzed by purified HIV-1 protease at 15 pM in buffer (50 mM sodium acetate, pH 5.5, 100 mM NaCl, 1 mg/mL bovine serum albumin) using a peptide substrate with sequence Val-Ser-Gln-Asn-(β-naphthylalanine)-Pro-Ile-Val at 440 μM in a final volume of 80 μL. Compounds in DMSO stock were added to a final DMSO concentration of 2.5% and preincubated with enzyme prior to the initiation of the reaction by addition of substrate. Inhibitor dose titrations were used to determine IC_{50} values. Reactions were incubated at 30 °C for 60 min and were then quenched by the addition of 120 μL of 10% H_3PO_4. The amount of product formed was determined using high performance liquid chromatography with a Vydac C18 column and fluorescence detection of product. Percent inhibition was determined relative to control samples without inhibitor, and IC_{50} values were determined using a standard four-parameter fit to the inhibition data. K_i values were determined from eq 1 for competitive inhibitors,

$$IC_{50} = K_i(1 + [S]/K_M) \quad (1)$$

with a K_M of 90 μM.

X-ray Crystallography. Crystallographic data for **25** have been deposited with the Cambridge Crystallographic Data Centre, deposition number CCDC 779587. The X-ray crystal structure of inhibitor **32** bound to HIV-1 protease has been deposited in the Protein Databank with access code 3TH9. The triple mutant Q7K L33I L63I was used because of its higher stability against autoproteolysis.[8] HIV-1 protease crystals were grown by the hanging-drop vapor diffusion method against 0.5 M NaCl, acetate buffer, pH 5.5. Plate-shaped crystals were soaked in the reservoir solution in the presence of 1 mM inhibitor **32**. 25% glycerol was added for cryoprotection, and crystals were flash-frozen in liquid nitrogen. Crystals were diffracted to 1.34 Å resolution, and a 97.5% complete data set was collected at the APS beamline ID17 (Argonne, IL) and processed using autoProc.[9] R_{merge} and I/σ were 3.5% and 23.4 for the whole data set and were 48.9% and 2.8 for the highest resolution shell, respectively. Data were reduced to space group $P2_12_12$ with unit cell dimensions of a = 58.6 Å, b = 85.9 Å, c = 46.2 Å and one dimer per asymmetric unit. The structure was refined using Buster[10] and refitted using Coot,[11] resulting in final R_{work} (R_{free}) of 18.0% (19.1%) and excellent geometry. Figures were generated using Pymol.[12]

Accession Codes

[+]The PDB code for HIV-1 protease complex with **32** is 3TH9.

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ABBREVIATIONS USED

HIV, human immunodeficiency virus; AIDS, acquired immuno deficiency syndrome; K_i, absolute inhibition constant; IC_{50}, half maximal inhibitory concentration; AIBN, azobisisobutyronitrile; DCM, dichloromethane; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; TLC, thin layer chromatography

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