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Design and Synthesis of Piperazine Sulfonamide Cores Leading to Highly Potent HIV-1 Protease Inhibitors

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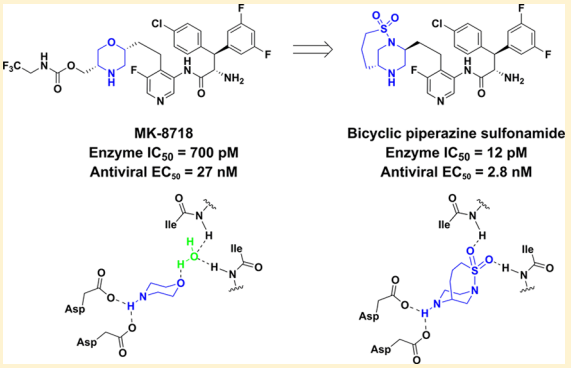
S *Supporting Information*

ABSTRACT: Using the HIV-1 protease binding mode of **MK-8718** and **PL-100** as inspiration, a novel aspartate binding bicyclic piperazine sulfonamide core was designed and synthesized. The resulting HIV-1 protease inhibitor containing this core showed an 60-fold increase in enzyme binding affinity and a 10-fold increase in antiviral activity relative to **MK-8718**.



KEYWORDS: *HIV-1 protease inhibitors, MK-8718, PL-100, piperazine sulfonamide*

HIV-1 protease is a critical enzyme in the lifecycle of the virus, serving to catalyze the proteolytic cleavage of polypeptide precursors into mature enzymes and structural proteins that are essential components of HIV-1.[1] Inhibitors of this enzyme prevent conversion of HIV-1 particles into their mature infectious form, and so it follows that HIV-1 protease inhibitors represent an important therapeutic approach for the treatment of HIV-1 infection.[2−4] Recently, we reported the discovery of **MK-8718**, an HIV-1 protease inhibitor containing a novel morpholine aspartate binding group.[5] A key feature of this inhibitor is that the morpholine amine forms the key interaction with the Asp-25$_A$ and Asp-25$_B$ acidic residues of the enzyme, in contrast to the majority of inhibitors where a hydroxyl group plays this role.[6] Herein we report further optimization of the enzyme bound conformation of these amine based class of inhibitors.

Inspiration for the design of our next generation inhibitors came from examining the enzyme bound conformations of **MK-8718**[5] and **PL-100**[7] (Figure 1). It can be seen that the morpholine oxygen of **MK-8718** binds to the flap of the enzyme (Ile50$_A$ and Ile50$_B$) via a bridging water. In contrast, the sulfonamide moiety present in **PL-100** binds directly to the Ile50$_A$ and Ile50$_B$ residues. This observation led us to design the hybrid core shown below in Figure 1. The so-designed piperazine sulfonamide would retain the amine to form the key interaction with the Asp-25$_A$ and Asp-25$_B$ acidic residues, while the sulfonyl group would displace the bridging water and bind directly to the flap Ile50$_A$ and Ile50$_B$ residues. In order to simplify the synthesis of the initial proof of principal target, a simplified right-hand side derived from 3,3-bis(4-fluorophenyl)propanoic acid was utilized.[8] Synthesis of our initial design is outlined in Scheme 1. Commercially available racemic **1** was sulfonylated to afford piperazine sulfonamide **2**. Dess−Martin oxidation[9] gave aldehyde **3**, which underwent Wittig olefination using (2-nitrobenzyl)triphenylphosphonium bromide[10] to afford olefin **4**. Concomitant reduction of the nitro and olefin functionalities under hydrogenation conditions yielded aniline **5**. Coupling of 3,3-bis(4-fluorophenyl)-propanoic acid with aniline **5** afforded amide **6**. Elaboration to the desired target was achieved by Boc-deprotection and resolution of the ensuing enantiomers to afford **7** and **8**. Antiviral activity of both enantiomers **7** and **8** was measured, and pleasingly,

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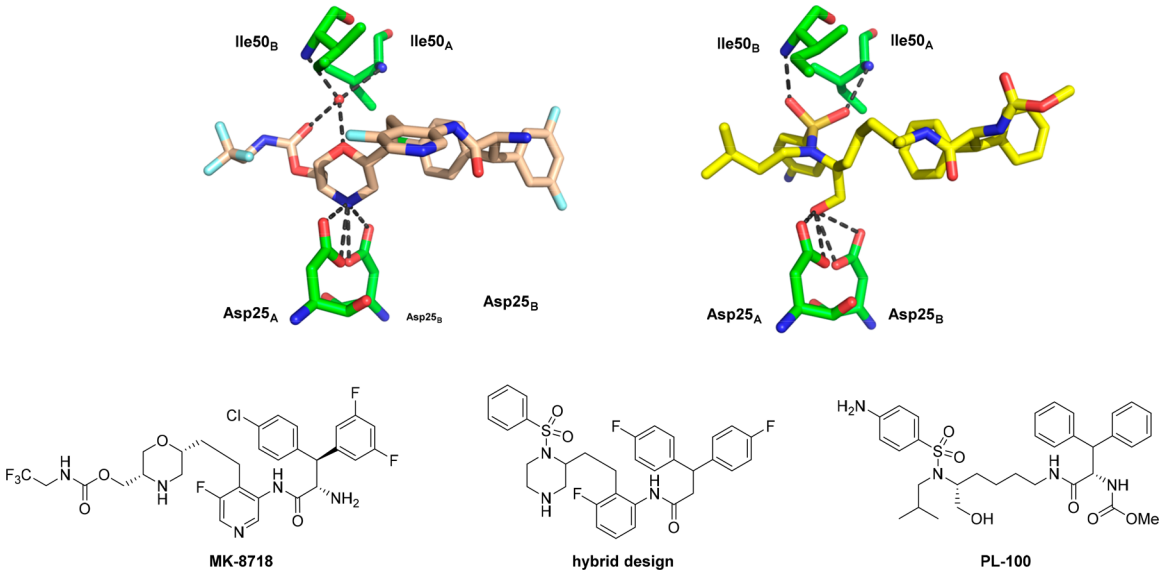
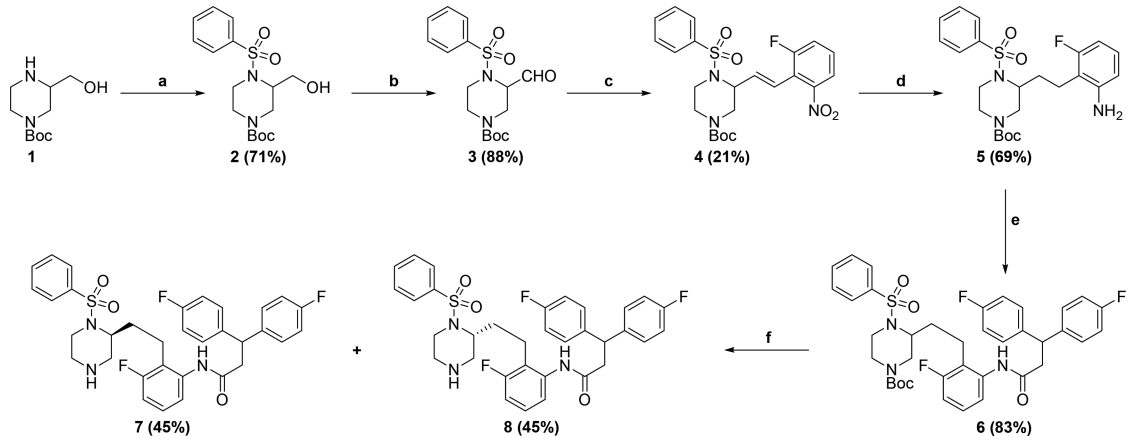


Figure 1. Hybrid design concept based on the binding modes of MK-8718 and PL-100 (PDB codes 5IVT and 2QMP).

Scheme 1[a]



[a]Reagents and conditions: (a) $PhSO_2Cl$, Hunig's base, CH_2Cl_2, −78 °C to RT; (b) Dess−Martin periodinane, CH_2Cl_2, 0 °C; (c) K_2CO_3, 18-*crown*-6, (2-nitrobenzyl)triphenylphosphonium bromide, DME, RT; (d) Pearlman's catalyst, H_2 balloon, CF_3CH_2OH, RT; (e) 3,3-Bis(4-fluorophenyl)propanoic acid, T3P, Hunig's base, EtOAc, RT; (f) TFA, CH_2Cl_2, RT, then Chiralpak AD.

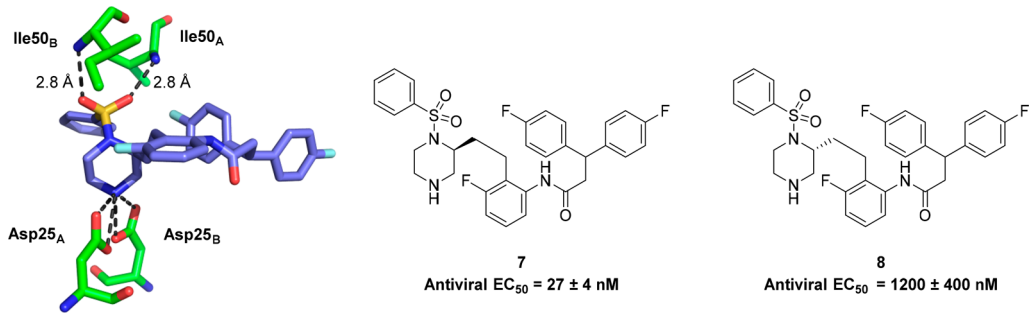


Figure 2. X-ray crystal structure of 7 bound to HIV-1 protease showing hydrogen bonding to $Ile50_A$ and $Ile50_B$ residues.

enantiomer 7 showed significant activity in a cell-based antiviral assay (EC_{50} = 27 nM).[11]

With this active compound in hand, we decided to pursue an X-ray crystal structure of 7 bound to HIV-1 protease to confirm our hypothesis that the sulfonamide moiety had displaced the water molecule present in the enzyme bound structure of **MK-8718**. Gratifyingly, the crystal structure of 7 bound to HIV-1 protease, shown in Figure 2, indeed showed that no bridging water was present, and the sulfone of 7 was directly hydrogen bonded to the $Ile50_A$ and $Ile50_B$ residues of the enzyme. Also evident from the crystal structure of 7 was that the preferred stereochemistry at the 2-position of the piperazine moiety was

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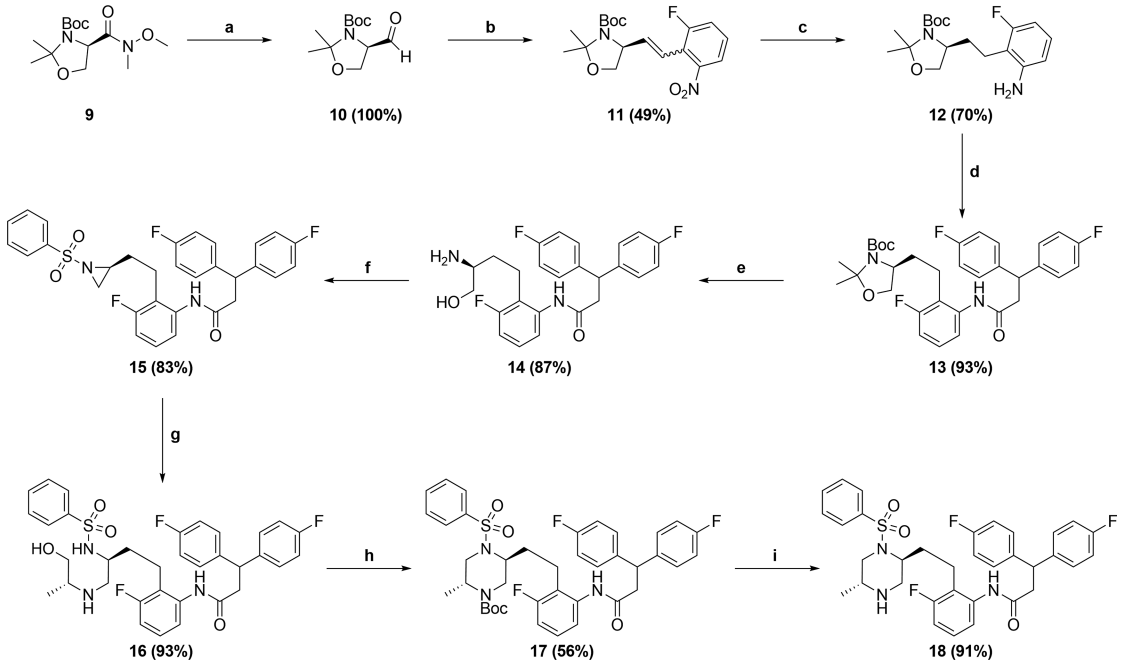
Scheme 2[a]



[a]Reagents and conditions: (a) $LiAlH_4$, 2-Me-THF, 0 °C; (b) K_2CO_3, 18-*crown*-6, (2-nitrobenzyl)triphenylphosphonium bromide, DME, RT; (c) Pearlman's catalyst, 50 psi H_2, EtOAc/MeOH, RT; (d) 3,3-Bis(4-fluorophenyl)propanoic acid, T3P, Hunig's base, EtOAc, RT; (e) TFA, H_2O, CH_2Cl_2; RT; (f) (i) $PhSO_2Cl$, NEt_3, DMF, 0 °C; (ii) diazene-1,2-diylbismorpholinomethanone, PBu_3, THF, RT; (g) (*R*)-2-aminopropan-1-ol, 1,2-DCE, 40 °C; (h) (i) Boc_2O, NEt_3, CH_2Cl_2, RT; (ii) diazene-1,2-diylbismorpholinomethanone, PBu_3, THF, RT; (i) TFA, CH_2Cl_2, RT.

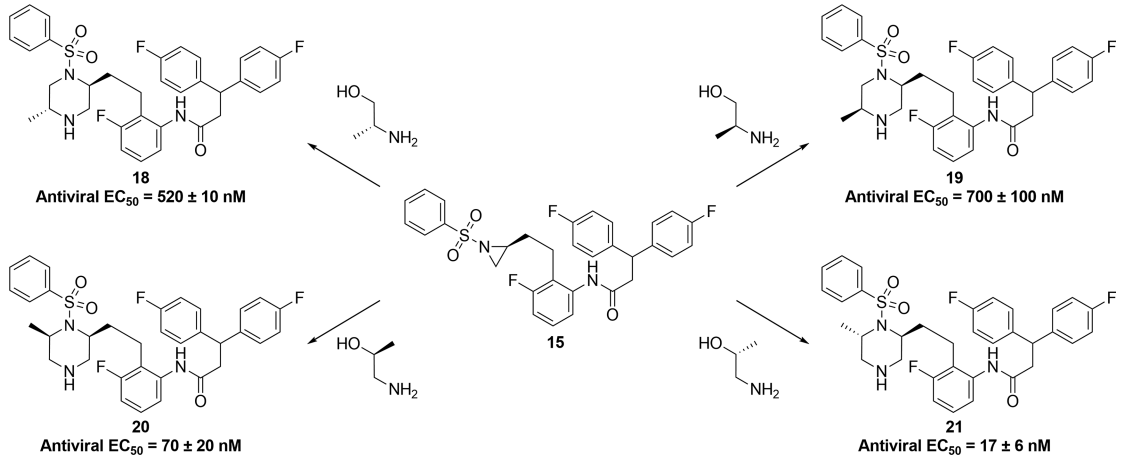


Figure 3. Antiviral activity (EC_{50})[11] of analogues **18**−**21** formed via amino alcohol opening of aziridine **15**.

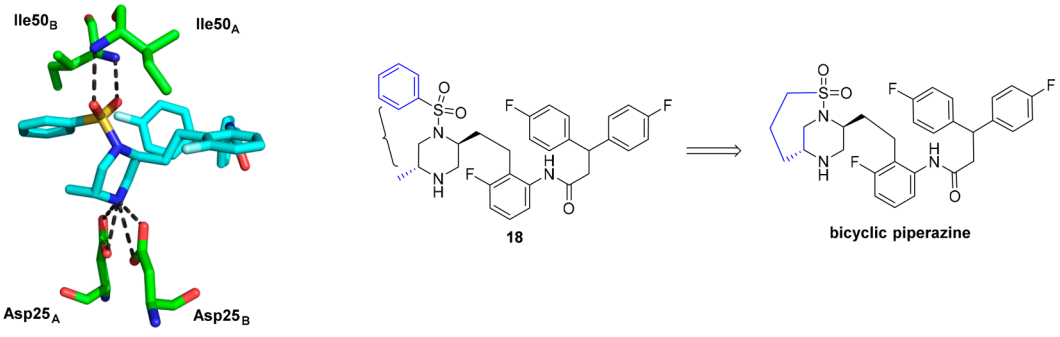


Figure 4. X-ray crystal structure of **18** bound to HIV-1 protease, showing steric clash and leading to design of a bicyclic piperazine.

the (*S*)-configuration, opposite to that which is preferred for the morpholine core of **MK-8718**. This was in concurrence with results from earlier modeling studies of the piperazine sulfonamide core, which showed the HIV-1 protease binding

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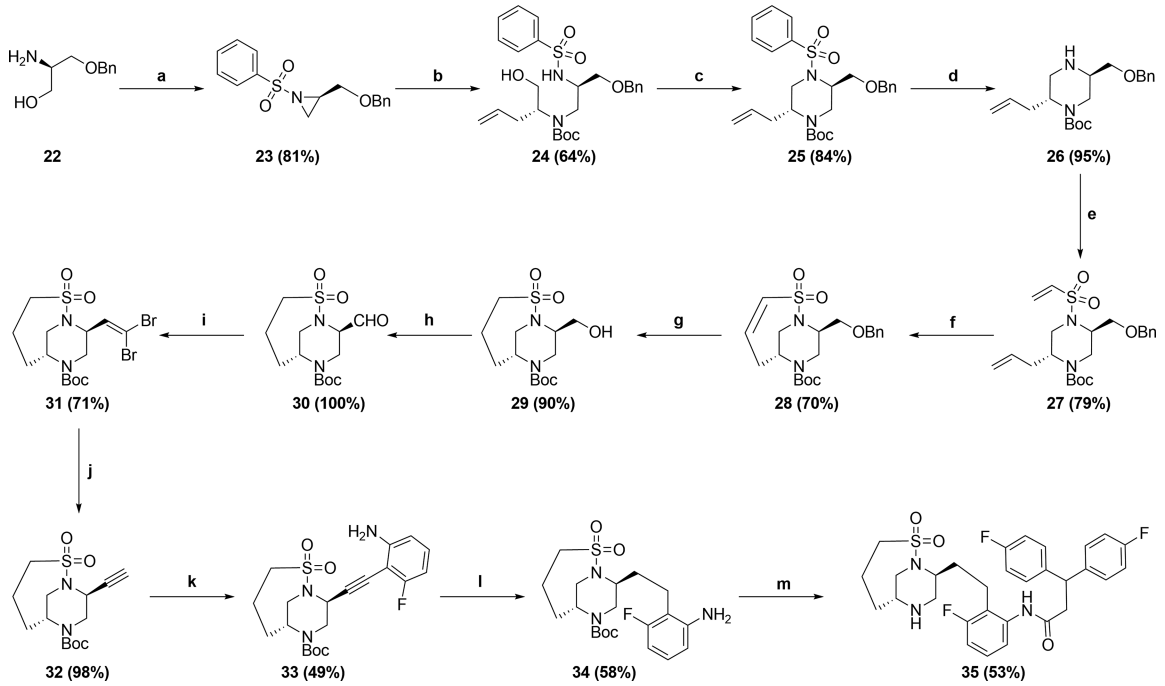
Scheme 3[a]



[a](a) (i) $PhSO_2Cl$, NEt_3, DMF, 0 °C; (ii) DIAD, PBu_3, THF, 0 °C; (b) (i) (*R*)-2-aminopent-4-en-1-ol, THF, 45 °C; (ii) Boc_2O, NEt_3, CH_3CN, 45 °C; (c) DIAD, PBu_3, THF, RT; (d) Mg, MeOH, sonication, RT; (e) 2-chloroethanesulfonyl chloride, NEt_3, CH_2Cl_2, RT; (f) Zhan Catalyst-1B, 1,2-DCE, 50 °C; (g) Pearlman's catalyst, H_2 balloon, EtOAc, RT; (h) Dess–Martin periodinane, CH_2Cl_2, RT; (i) PPh_3, CBr_4, CH_2Cl_2, RT; (j) EtMgBr, THF, 0 °C; (k) 3-fluoro-2-iodoaniline, $(PPh_3)_2PdCl_2$, CuI, NEt_3, CH_3CN, 70 °C; (l) Pearlman's catalyst, H_2 balloon, EtOH, RT; (m) (i) 3,3-Bis(4-fluorophenyl)propanoic acid, T3P, Hunig's base, EtOAc, RT; (ii) HCl, dioxane, RT.

conformation of the (*S*)-enantiomer to be lower in energy than that of the corresponding (*R*)-enantiomer.

Although piperazine sulfonamide **7** displayed good antiviral activity, *in vitro* metabolic studies revealed that the unsubstituted left-hand side of the piperazine ring was susceptible to significant metabolic oxidation. This prompted us to investigate whether functionalization of the unsubstituted side of the piperazine ring would be tolerated with respect to antiviral activity. In order to introduce a methyl group on the left-hand side of the piperazine in a regio- and stereocontrolled manner, the route shown in Scheme 2 was utilized.[12] The route starts with readily available and configurationally stable Weinreb amide **9**. Reduction of **9** afforded aldehyde **10**,[13] which underwent Wittig olefination to give **11**. Reduction of both the olefin and nitro groups afforded aniline **12**, which was coupled with 3,3-bis(4-fluorophenyl)-propanoic acid to afford amide **13**. Deprotection of **13** afforded amino alcohol **14**, which was converted to aziridine **15** via a sulfonylation and subsequent intramolecular Mitsunobu[14] reaction. Opening of aziridine **15** with (*R*)-2-aminopropan-1-ol, followed by Boc-protection afforded **16**, which was converted to piperazine **17** via an intramolecular Mitsunobu reaction. Boc-deprotection afforded the desired target **18**. Aziridine **15** was used to synthesize all four methyl-substituted isomers as shown in Figure 3.

Although **20** and **21** with methyl substituents at the 6-position of the piperazine showed similar potency to unsubstituted piperazine **7**, we were intrigued by the X-ray crystal structure of the less potent analogue **18** bound to HIV-1 protease, as shown in Figure 4. It can be seen that the phenyl group on the sulfonamide moiety and the methyl group on the piperazine ring lie in close proximity to each other, likely creating an unfavorable steric interaction in the binding conformation of the molecule. This observation led us to consider forming a bicyclic ring, with a bond joining the sulfone to the piperazine ring. Molecular modeling suggested a three carbon chain length would be optimal for locking the molecule in the bioactive conformation. Synthesis of this target is shown in Scheme 3 and began with commercially available amino alcohol **22**. Aziridine formation via sulfonylation and Mitsunobu ring closure afforded intermediate **23**. Aziridine **23** was opened with (*R*)-2-aminopent-4-en-1-ol, and subsequent Boc-protection yielded **24**. An intramolecular Mitsunobu reaction gave piperazine **25**, and subsequent magnesium/MeOH mediated deprotection[15] cleanly removed the phenylsulfonyl moiety in the presence of both the *N*-Boc and *O*-Bn moieties to give **26**. Sulfonylation of **26** gave metathesis precursor **27**, which underwent ring closure mediated by Zhan Catalyst-1B[16] to give bicyclic compound **28**. Hydrogenation of **28** reduced the olefin and removed the benzyl group yielding **29**. Dess–Martin oxidation, followed by Corey Fuchs alkyne formation[17] via dibromide **31**, afforded alkyne **32**. Sonogashira coupling,[18] followed by reduction of so-formed **33** afforded aniline **34**. Coupling 3,3-bis(4-fluorophenyl)propanoic acid with aniline **34** and subsequent Boc-deprotection gave the bicyclic target **35**. We were pleased to observe that bicyclic compound **35** retained good antiviral activity (EC_{50} = 21 nM).[11] The X-ray crystal structure of **35** bound to HIV-1 protease, as shown in Figure 5, revealed the expected binding mode, whereby the sulfonyl group forms hydrogen bonds directly to flap Ile50A and Ile50B residues, taking the place of the bridging water present in the crystal structure of **MK-8718** bound to HIV-1 protease.

With this new core in hand, we decided to incorporate the highly optimized pieces of **MK-8718** into a molecule. The synthesis was carried out starting with alkyne **32** as shown in Scheme 4. Sonogashira coupling, followed by reduction, afforded

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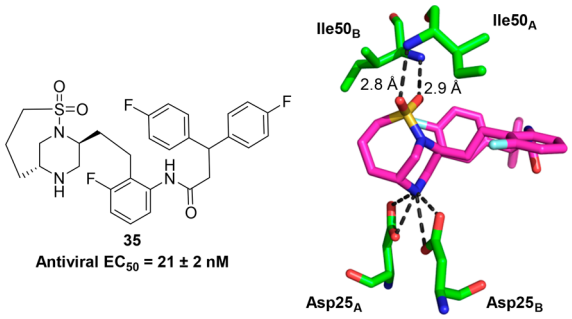
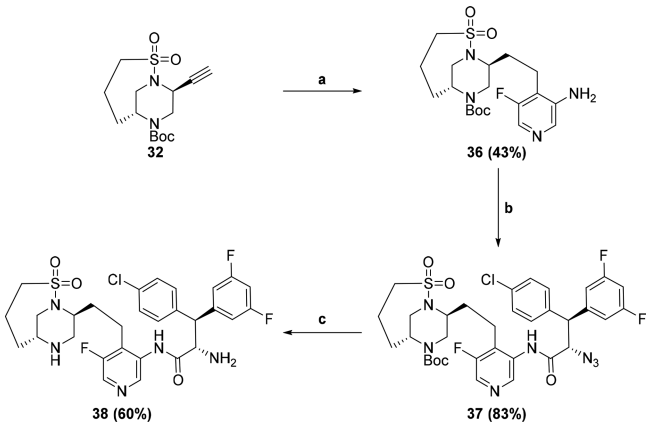


Figure 5. Antiviral activity (EC_{50})[11] and X-ray crystal structure of **35** bound to HIV-1 protease.

Scheme 4[a]



[a]Reagents and conditions: (a) (i) 5-fluoro-4-iodopyridin-3-amine, $(PPh_3)_2PdCl_2$, CuI, NEt_3, CH_3CN, 70 °C; (ii) Pearlman's catalyst, H_2 balloon, EtOH, RT; (b) (2*S*,3*S*)-2-azido-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoic acid, $POCl_3$, pyridine, 0 °C; (c) (i) PMe_3, THF/H_2O, 0 °C; (ii) HCl, dioxane/CH_2Cl_2, RT.

amine **36**. Coupling of amine **36** with (2*S*,3*S*)-2-azido-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoic acid[5] gave amide **37**, which, after azide reduction and Boc-deprotection produced the final compound **38**. This compound showed exquisite enzyme binding affinity (IC_{50} = 12 pM) for HIV-1 protease, which translated into potent antiviral activity (EC_{50} = 2.8 nM) in a cell-based assay. As shown in Table 1, this represents a significant

Table 1. Enzyme Affinity and Antiviral Activity of Key Molecules

compound	enzyme[19] IC_{50} (pM)	antiviral[11] EC_{50} (nM)
MK-8718	700 ± 600	27 ± 7
38	12 ± 1	2.8 ± 0.4
Atazanavir	40 ± 30	12 ± 4
Darunavir	13 ± 3	7 ± 2

potency improvement relative to **MK-8718** (binding IC_{50} = 700 pM, antiviral EC_{50} = 27 nM). In addition, the potency of **38** compares favorably to market leading HIV-1 protease inhibitors Atazanavir and Darunavir.

In summary, through a series of structure-based design iterations, a novel bicyclic piperazine sulfonamide aspartyl protease binding core was identified. This core produced a 60-fold increase in HIV-1 protease binding affinity and a 10-fold increase in antiviral activity relative to **MK-8718**.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00386.

Synthetic experimental details for the synthesis of **7**, **8**, **18**−**21**, and **35**, descriptions of primary biological assays, procedures for cocrystallization studies, and a table of crystallographic statistics, along with *in vivo* rat pharmacokinetic data for **7**, **18**, **20**, and **21** (PDF)

Accession Codes

X-ray crystallographic data for **7**, **18**, **19**, **20**, **21**, and **35** bound to HIV-1 protease have been deposited in the RCSB protein data bank as 6B36, 6B3C, 6B3F, 6B38, 6B3G, and 6B3H, respectively.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

HIV, human immunodeficiency virus; Ile, isoleucine; Asp, aspartic acid; Boc, *tert*-butyloxycarbonyl; 1,2-DCE, 1,2-dichloroethane; DIAD, diisopropyl azodicarboxylate; DME, 1,2-dimethoxyethane; DMF, dimethylformamide; EtOAc, ethyl acetate; MeOH, methanol; NEt_3, triethylamine; PBu_3, tributylphosphine; RT, room temperature; T3P, 1-propanephosphonic anhydride; THF, tetrahydrofuran; TFA, trifluoroacetic acid.

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(19) Assay for inhibition of HIV-1 protease as described in the Supporting Information; potency is reported as an IC_{50} (average of at least $n = 2$ runs).

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