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Design, Synthesis, and Biological Evaluation of HIV/FIV Protease Inhibitors Incorporating a Conformationally Constrained Macrocycle with a Small P3′ Residue

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Abstract—A series of norstatine-based HIV/FIV protease inhibitors incorporating a 15-membered macrocycle as a mimic of the tripeptide (Ala-Val-Phe), a motif with a small P3′ residue effective against the FIV protease and the drug-resistant HIV proteases, has been synthesized. It was found that the macrocycle is important to the overall activity of the inhibitors. Certain inhibitors were developed expressing low nanomolar inhibitory activity against the HIV/FIV proteases and they are also effective against some drug-resistant as well as TL3-resistant HIV proteases. © 2001 Elsevier Science Ltd. All rights reserved.

Despite the approval of several inhibitors of the HIV protease,[1,2] many drug-resistant variants of HIV have been identified.[2–4] Feline immunodeficiency virus (FIV) causes an immunodeficiency syndrome in cats comparable to AIDS in humans. Although the active site structures of HIV and FIV proteases are superimposable and have an identical mechanism of catalysis,[5,6] all the approved inhibitors, with K_i values in the low nanomolar range for HIV protease, only bind to the FIV protease in the micromolar range. FIV protease has a more restricted P3 binding subsite than does the wild-type HIV protease and resembles many drug-resistant HIV proteases that were found to have a shrunk P3 binding subsite;[7] good inhibitors of the FIV protease with small P3 residue, especially Ala, were thus shown to be better inhibitors of the wild-type and drug-resistant HIV proteases.[7] For example, compound **1** (TL3) with Ala residue in P3 positions demonstrated nanomolar inhibitions in wild-type FIV/HIV proteases and some drug-resistant HIV mutants. Also **1** showed strong ability to inhibit replication of FIV, HIV, and SIV with virtually the same degree of effectiveness (~1 μM) in tissue culture.[7] FIV protease therefore has been considered as the drug-resistant phenotype of HIV protease and used as a model for the development of new inhibitors to tackle the problem of drug resistance.

It has been demonstrated that the introduction of macrocyclic structures in HIV protease inhibitors can improve their binding affinity and resistance toward proteolytic enzymes.[8–14] In addition, HIV protease inhibitors containing the norstatine analogue (3-amino-2-hydroxy-4-phenylbutyric acid),[15–18] particularly **2** (JE-2147) (Fig. 1), have very potent antiviral activities in vitro and exhibit good oral bioavailability and plasma pharmacokinetic profiles. These reports have prompted us to disclose our approach to the design, synthesis, and in vitro evaluation of a series of norstatine-based macrocyclic inhibitors **3**–**7** (Fig. 1). The macrocycle was designed to mimic the conformationally constrained Ala-Val-Phe motif which, with Ala in the P3 position, was used in the development of FIV and drug-resistant HIV protease inhibitors. We have found that several of these structures inhibit the FIV and drug-resistant HIV enzymes at low nanomolar concentrations.

Results and Discussion

The X-ray structure of FIV protease complexed with **1**[19] reveals that the P1 and P3 residues are positioned very

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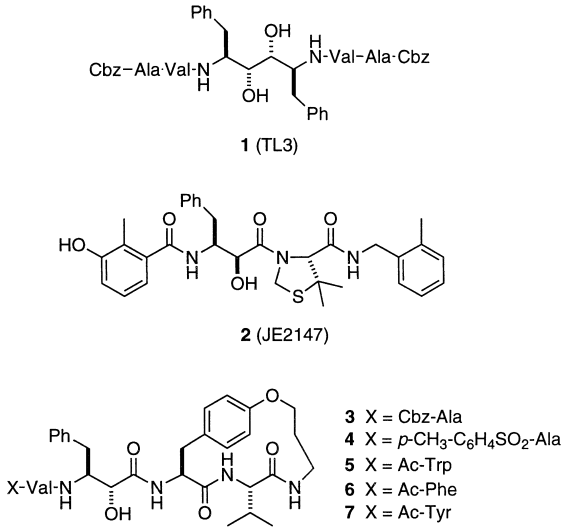
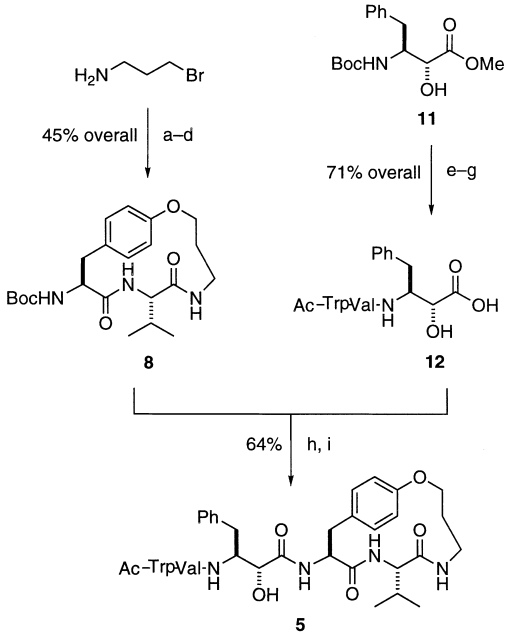


Figure 1.

closely in the neighboring hydrophobic pockets. Connecting the P1 and P3 residues with the aliphatic chain in a macrocycle may render the solution conformation similar to the bound conformation in the active site of the protease. With this speculation, we decided to choose the macrocycle **8** (Scheme 1) as a mimic of the tripeptide (Ala-Val-Phe)[11–13] in our macrocyclic protease inhibitors.

The configuration of the C-OH stereogenic center in the norstatine-based protease inhibitors is known to be important for the inhibitory activity.[15–18] Specifically, compound **9** with *R* stereochemistry around its essential carbinol function was found to have better inhibitory activity (~6-fold) for HIV/FIV proteases than that of



Scheme 1. Reagents and conditions: (a) Boc-Val-OH, HBTU, DIPEA, THF; (b) TFA, CH_2Cl_2; (c) Boc-Tyr-OH, HBTU, DIPEA, THF; (d) $CsCO_3$, TBAI, CH_3CN; (e) TFA, CH_2Cl_2; (f) Ac-Trp-Val-OH, HBTU, DIPEA, THF; (g) LiOH, MeOH/H_2O; (h) TFA, CH_2Cl_2; (i) HBTU, DIPEA, DMF.

the *S* isomer **10** (Fig. 2). In addition, replacement of Cbz in TL3 with *para*-toluenesulfonyl protecting groups increases the activity (~5-fold) toward FIV protease.[20] As a result, extending the main chain in the *N*-terminus of **9** with Z-Ala-Val-OH and *p*-CH_3-$C_6H_4SO_2$-Ala-Val-OH to produce **3** and **4**, respectively, is our initial approach. Dunn et al.[21] have also reported that nanomolar inhibition for FIV protease can be achieved by capping the *N*-terminus with the small acetyl protecting group together with a relatively large side-chain residue such as naphthalene methyl in the P3 and P3′ positions. Modeling of these inhibitors complexed with the enzyme revealed the naphthalene group has moved from the P3 or P3′ binding subsites toward the outside of the cleft. Based on this observation, we also have designed compounds **5**–**7** containing the dipeptides Ac-Trp-Val-OH, Ac-Phe-Val-OH and Ac-Tyr-Val-OH as the P3 and P2 residues.

Our most efficient synthetic route toward our inhibitors **3**–**7** is illustrated in Scheme 1, as described for cyclic inhibitor **5**. The synthesis involves the preparation of the hydroxyl acid **12** derived from **11**,[22] followed by incorporation of the macrocycle **8** after Boc removal. Macrocyclization of **8** was achieved efficiently by the treatment of Cs_2CO_3 in CH_3CN (10 mM) in the presence of 1 equiv of tetrabutylammonium iodide at room temperature. Despite introduction of the flexible trimethylene ether linker, the NMR spectrum indicates four unequivalent aromatic protons in the cycle, suggesting that the 15-membered ring system is highly constrained and the aromatic ring is not able to rotate freely.

The inhibitory activities of each compound against FIV, HIV, drug-resistant mutant HIV, and TL3-resistant mutant HIV proteases were determined as described previously,[7] and the results are summarized in Table 1.

The results revealed that **3** and **4** have similar potency against HIV/FIV proteases though they are slightly less potent than TL3. However the inhibitors **5**–**7** showed a comparable activity to TL3 against HIV protease. Compounds **5**–**7** also exhibited remarkable activities toward FIV protease with IC_{50} values about 2- to 3-fold lower than TL3. In particular, **7** was found to be the most potent cyclic inhibitor of FIV protease reported to date, with an IC_{50} value of only 27 nM.

Although TL3 displayed a significant inhibitory activity against HIV/FIV and some drug-resistant HIV proteases, its development of resistance has been studied

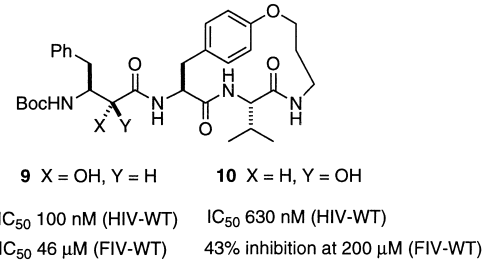


Figure 2.

Table 1. Biological activity (IC_{50} nM) of TL3 and **3**–**7** against FIV-WT, HIV-WT, drug-resistant HIV and TL3-resistant HIV proteases

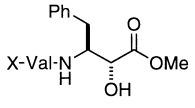
			Drug-resistant HIV mutants		TL3-resistant HIV mutants	
	FIV PR	HIV PR	HIV (G48V)	HIV (V82F)	HIV (V82A)	HIV (L24I, M46I, F53L, L63P, V77I, V82A)
Inhibitors	IC_{50} (nM)					
TL3	72	4	20.5 (5×)[a]	14.9 (4×)	16 (4×)	144 (36×)
3	95	10	nd[b]	nd	nd	nd
4	147	13	nd	nd	nd	nd
5	49	10	71 (7×)	30 (3×)	10 (1×)	49 (5×)
6	35	7	94 (13×)	32 (4×)	12 (2×)	79 (11×)
7	27	6	57 (9.5×)	26 (4×)	11 (2×)	70 (12×)

[a]Number in parentheses denotes number of fold higher IC_{50} values in inhibition compared to that of the wild-type HIV PR.
[b]Not determined.

under forcing conditions and TL3-resistant HIV proteases with up to six mutations have been identified. Since inhibitors **5**–**7** showed better activity toward FIV protease as compared to TL3, the inhibitory activities of **5**–**7** were also evaluated against several drug-resistant and TL3-resistant HIV proteases. All these mutant enzymes have their mutated residues affect the S3 and S3′ subsites, which have been identified as the important areas associated with the development of drug resistance.[7]

Compared to TL3, which is 5-fold weaker toward the drug-resistant mutant G48V, compounds **5**–**7** are less effective against this mutant, and similarly effective against the V82F mutant. In the case for TL3-resistant mutants, the results were also noteworthy. The cyclic inhibitors **5**–**7**, in general, have higher potency than that of TL3. In particular, **5**–**7** showed an improved inhibitory activity against the hexa-mutant induced by TL3. These results indicate that inhibitors with the designed cyclic motif such as **5**–**7** may also be effective against a broad range of HIV variants.

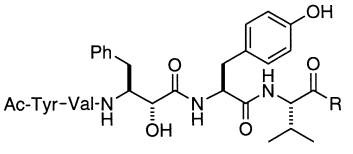
The macrocycle was found to be important for the overall activity of the inhibitors, as the hydroxyl methyl esters **13**–**15** (Fig. 3) were found to be less potent than that of **9** against HIV/FIV proteases. Also, hexapeptide **16**, which is the acyclic analogue of **7**, displayed about 20- or 35-fold higher IC_{50} values for wild-type FIV and HIV PRs, respectively, as compared to **7**. Such phenomena may be



13 X = Ac-Trp IC_{50} 14 μM (HIV-WT)
IC_{50} 66 μM (FIV-WT)

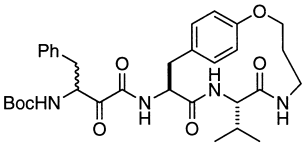
14 X = Ac-Phe IC_{50} 113 μM (HIV-WT)
24% inhibition at 200 μM (FIV-WT)

15 X = Ac-Tyr IC_{50} 71 μM (HIV-WT)
32% inhibition at 200 μM (FIV-WT)



16 R = NH_2 IC_{50} 212 nM (HIV-WT)
IC_{50} 560 nM (FIV-WT)

Figure 3.



17

IC_{50} 13 nM (HIV-WT)
IC_{50} 4 μM (FIV-WT)

Figure 4.

attributed to the macrocycle better pre-organized in a favorable enzyme-bound conformation, resulting in an entropy advantage gained over the acyclic compound.[11–14]

It is also interesting to note that the keto amide **17** (Fig. 4) is about 10-fold more active than the corresponding hydroxyl amide **9** against HIV/FIV proteases.[5,23] Oxidation of **5**–**7** to the corresponding keto amide analogues may generate a better series of protease inhibitors.

In summary, we have described a general strategy to introduce a structural and functional mimic of the tripeptide (Ala-Val-Phe) in the form of a 15-membered ring into our protease inhibitors. Certain inhibitors were developed expressing low nanomolar HIV/FIV protease inhibitory activity. Bicyclic inhibitors incorporating two similar macrocycles are currently under investigation and will be reported in due course.

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