Brief Articles

Reduction of Peptide Character of HIV Protease Inhibitors That Exhibit Nanomolar Potency against Multidrug Resistant HIV-1 Strains

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Novel HIV protease inhibitors containing a hydroxyethylamine dipeptide isostere as a transition state-mimic king structure were synthesized by combining substructures of known HIV protease inhibitors. Among them, TYA5 and TYB5 were proven to be not only potent enzyme inhibitors (K_i = 0.12 nM and 0.10 nM, respectively) but also strong anti-HIV agents (IC_{50} = 9.5 nM and 66 nM, respectively), even against viral strains with multidrug resistance. Furthermore, insertion of an (*E*)-alkene dipeptide isostere at the P_1-P_2 position of TYB5 led to development of a purely nonpeptidic protease inhibitor, TYB1 (K_i = 0.38 nM, IC_{50} = 160 nM).

Introduction

Disclosure of the molecular mechanism relevant to each step of the HIV-1 life cycle has led to development of many types of anti-HIV agents. The multiple drug-combination chemotherapy, "highly active anti-retroviral therapy (HAART)", which involves a combination of reverse transcriptase inhibitors and protease inhibitors,[1−9] has dramatically improved the clinical treatment of individuals with HIV-infection or AIDS. However, there still remain several serious problems including the emergence of viral strains with multidrug resistance, significant adverse effects and high costs.[10] Many HIV protease inhibitors also have the disadvantage of low bioavailability due to their peptide character. All HIV protease inhibitors that have been clinically used to date, contain one or more amide bonds. Discovery and development of novel potent HIV protease inhibitors that are active against multidrug resistant viral strains and have excellent biostability and bioavailability, are required for HAART. By combining substructure units of known HIV protease inhibitors, we have derived novel potent inhibitors that are effective even against multidrug resistant viruses. Subsequently, in an effort to improve bioavailability by developing inhibitors that contain no amide bonds, an (*E*)-alkene dipeptide isostere (EADI)[11] was introduced into the above inhibitors. The 3D structure of this (*E*)-alkene peptide mimic (bond length, bond angle, and rigidity) closely resembles that of the parent amide.

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Chemistry

The efficacy of hydroxyethylamine dipeptide isosteres (HDIs)[12] as backbone replacements of amide bonds in the position $P_1-P_{1'}$ of aspartyl protease inhibitors has been well documented. Several target compounds containing an HDI transition state-mimic were designed based on structural information derived from reported HIV protease inhibitors. Among them, TYA5 (**7**) has a decahydroisoquinoline unit, which was used in saquinavir[2,3] and nelfinavir,[7] at the $P_{1'}-P_{2'}$ position, and a D-*S*-(2-naphthyl)cysteine unit, which was used in Lilly's compounds,[13] at the P_2-P_3 position (Figure 1). TYB5 (**14**) has a sulfonamide unit, which was used in amprenavir,[8] at the $P_{1'}-P_{2'}$ position, and a D-*S*-(2-naphthyl)cysteine unit at the P_2-P_3 position. Starting with a known, optically active epoxy amine **1**,[3,14−16] TYA5 (**7**) and TYB5 (**14**) were synthesized as diastereomeric sufoxides using general procedures illustrated in Scheme 1 (see Supporting Information). Target compounds **23a**,**b** (TYA1 and TYA2), in which the amide bonds at the P_1-P_2 position of compound **7** were replaced by (*E*)-alkenes, were synthesized using the (D-Ser, L-Phe)-type EADI [Cbz-D-Ser-ψ[(*E*)-CH=CH]-L-Phe-OBu^t] **15** (see Supporting Information)[17] according to Scheme 2 in company with their sulfone derivatives **24a**,**b** (TYA3 and TYA4). Stereochemistry at the C_2-carbon of diastereomers **23a**,**b** and **24a**,**b** has yet to be determined. Compound **29a** (TYB1), in which the amide bond at the P_1-P_2 position of compound **14** was replaced by an (*E*)-alkene, and its 2*R* isomer **29b** (TYB2) were synthesized using epoxy compounds **20a**,**b** according to Scheme 3. Stereochemistry at the C_2-carbon of diastereomers **29a**,**b** is based on X-ray analysis of **27b**. **23a**,**b** and **29a**,**b** are all comprised of sufoxide diastereomixtures.

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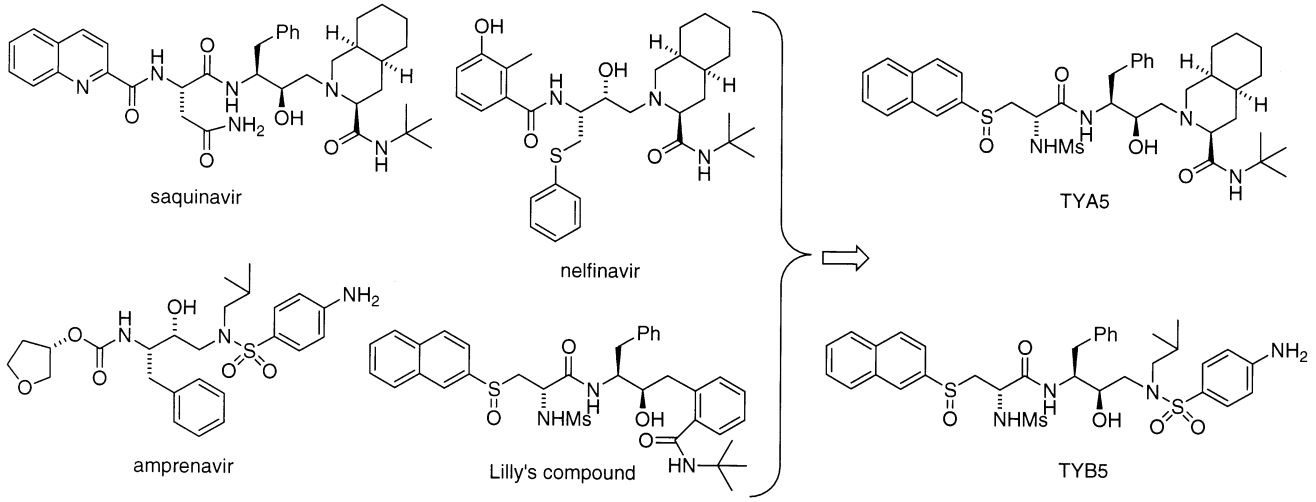
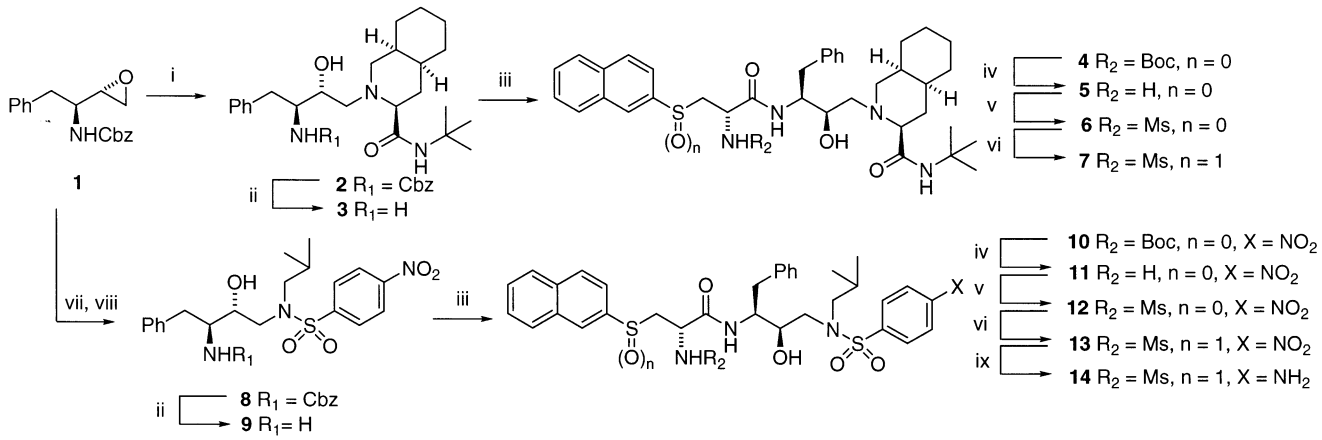


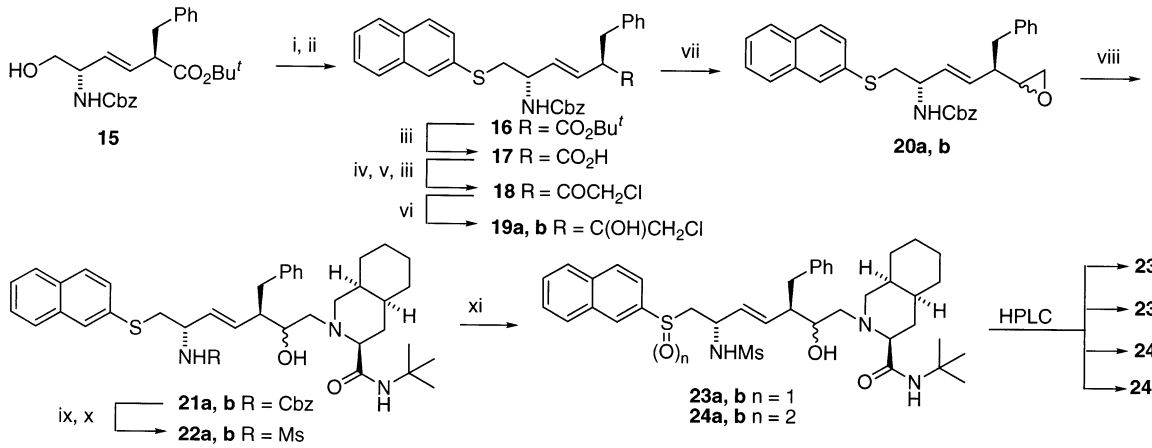
Figure 1. Structures of saquinavir, nelfinavir, amprenavir, Lilly's compound and our initial target compounds, TYA5 and TYB5.

Scheme 1[a]



[a] Reagents: (i) *N*-(*tert*-butyl)-decahydroisoquinolinecarboxamide; (ii) 1 M TMSBr-thioanisole/TFA; (iii) *N*-Boc-D-*S*-(2-naphthyl)cysteine, BOP, DIPEA, HOBt; (iv) 4 M HCl in 1,4-dioxane; (v) MsCl, DIPEA; (vi) $NaIO_4$; (vii) isobutylamine; (viii) *p*-nitrobenzenesulfonyl chloride, Et_3N; (ix) Zn, AcOH.

Scheme 2[a]

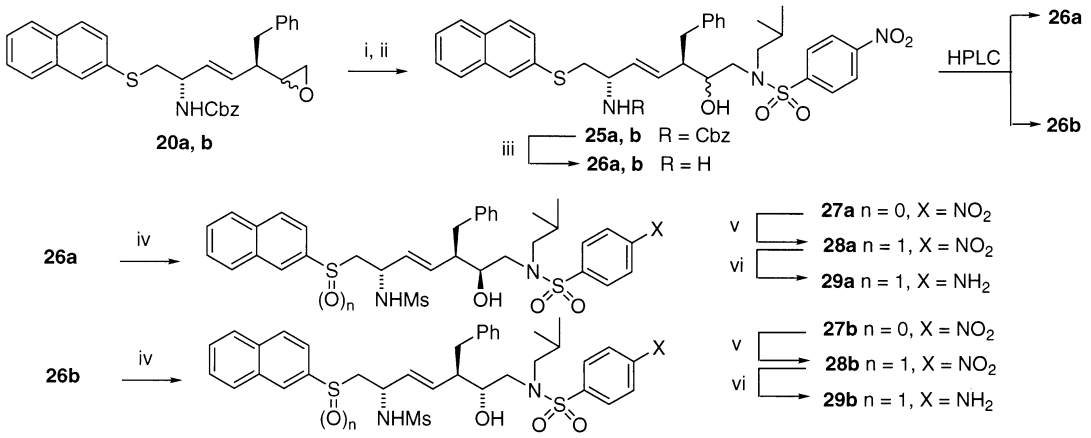


[a] Reagents: (i) TsCl, pyridine; (ii) 2-naphthalenethiol, NaH; (iii) 4 M HCl in 1,4-dioxane; (iv) isobutylchloroformate, DIPEA; (v) CH_2N_2; (vi) $NaBH_4$, H_2O; (vii) 0.5 M KOH in EtOH; (viii) *N*-(*tert*-butyl)-decahydroisoquinolinehydroxamide; (ix) TFA, thioanisole; (x) MsCl, DIPEA; (xi) $NaIO_4$.

Biological Results and Discussion

The anti-HIV activity of compounds, TYA5 and TYB5, was determined based on the inhibition of HIV-1-induced cytopathogenicity in MT-2 cells (described in Supporting Information).[18] TYA5 showed potent anti-HIV activity (Table 1). This potency was greater than those of saquinavir and amprenavir, which have been clinically used. TYB5 also showed potent activity. It was

Scheme 3[a]



[a] Reagents: (i) isobutylamine; (ii) *p*-nitrobenzenesulfonylchloride, Et_3N; (iii) TFA, thioanisole; (iv) MsCl, DIPEA; (v) $NaIO_4$; (vi) Zn, AcOH.

Table 1. Anti-HIV Activity, Cytotoxicity, and HIV Protease Inhibitory Activity of the Synthetic Compounds

compd (no.)	IC_{50} (nM)[a]	CC_{50} (μM)[b]	SI[c]	K_i (nM)[e]
TYA1 (**23a**)	>1000	NT		NT
TYA2 (**23b**)	>1000	NT		NT
TYA3 (**24a**)	>1000	NT		NT
TYA4 (**24b**)	>1000	NT		NT
TYA5 (**7**)	9.5 ± 0.3	3.9 ± 0.19	410	0.12
TYB1 (**29a**)	160 ± 40	8.0 ± 1.1	58	0.38
TYB2 (**29b**)	>1000	NT		NT
TYB5 (**14**)	66 ± 2	33 ± 12	500	0.10
AZT	27 ± 9	>100		NT
saquinavir	17 ± 3	11 ± 3	650	0.19
amprenavir	36 ± 11	>100		NT
indinavir	NT[d]	NT		0.28

[a] IC_{50} values are based on the inhibition of HIV-induced cytopathogenicity in MT-2 cells. [b] CC_{50} values are based on the reduction of the viability of mock-infected MT-2 cells. All data with standard deviation are the mean values for at least three independent experiments. Data without standard deviation are derived from the value for one experiment. [c] Selectivity index (SI) is shown as CC_{50}/IC_{50}. [d] NT: not tested. [e] K_i values are based on HIV-1 protease inhibitory activity.

noted that both TYA5 and TYB5 exhibited high selectivity indexes (SIs) as comparable to that of saquinavir.

Next, the anti-HIV activities of TYA5 and TYB5 were determined against multidrug resistant (MDR) strains as measured by the inhibition of HIV p24 antigen expression in peripheral blood mononuclear cells (PBMC) (described in Supporting Information).[18] TYB5 showed nearly equivalent activity against three MDR strains as against a wild-type strain, HIV_{104pre}. A reverse transcriptase inhibitor, AZT, and the reported protease inhibitors, saquinavir and amprenavir, showed 12−40-fold reduced potency against the three MDR strains as compared to against HIV_{104pre} (Table 2). TYA5 also exhibited effective activity (half potency) against MDR strains, except for HIV_{JSL}. The structure of TYA5 was based on the combination of a decahydroisoquinoline unit derived from saquinavir and a D-*S*-(2-naphthyl)-cysteine unit used in Lilly's compounds. The TYB5 structure was derived by combining a sulfonamide unit from amprenavir with a D-*S*-(2-naphthyl)cysteine unit. TYA5 and TYB5 are effective against MDR strains, while saquinavir and amprenavir exhibited low efficacy. By combining structural subunits from known inhibitors, protease inhibitors with high anti-HIV activity including against MDR HIV-1 have resulted.

Table 2. Anti-HIV Activity of TYA5, TYB5, and TYB1 against HIV-1 Clinical Isolates

		IC_{50} (nM) (fold change)[a] MDR[b]		
compd (no.)	wild type HIV_{104pre}	HIV_{TM}	HIV_{MM}	HIV_{JSL}
TYA5 (**7**)	15 ± 2	34 ± 4 (2×)	32 ± 4 (2×)	220± 40 (15×)
TYB5 (**14**)	31 ± 3	43 ± 6 (1×)	36 ± 8 (1×)	25 ± 6 (1×)
TYB1 (**29a**)	180 ± 30	>1000 (>6×)	520 ± 120 (3×)	340 ± 30 (2×)
AZT	2.5 ± 0.7	43 (17×)	37(15×)	64 (26×)
saquinavir	19 ± 4	230 ± 20 (12×)	320 ± 2 (17×)	550 (29×)
amprenavir	20 ± 3	480 (24×)	530 (27×)	800 (40×)

[a] IC_{50} values are based on the inhibition of HIV p24 antigen expression in PBMC. All data with standard deviation are the mean values for at least three experiments. Data without standard deviation are derived from the value for one experiment. [b] Amino acid substitutions in the protease-encoding region are shown in Supporting Information.

Finally, work was undertaken to reduce the peptide character of TYA5 and TYB5. TYB1, an amide bond mimic-containing compound based on TYB5, having syn geometry as in TYB5, showed moderate anti-HIV activity. Alternatively, TYA1−4, based on TYA5, did not show significant activity (Table 1). (Either TYA1 or TYA2 and either TYA3 or TYA4 are the same syn isomers as TYA5.) It is reasonable that the anti isomer, TYB2, did not show significant activity, whereas it was not readily explainable why the anti-HIV activities of compounds having amide bond mimics are less than those of the corresponding amide-containing compounds. Nonetheless, the anti-HIV activity of TYB1 was noted. As such, the HIV protease inhibitory activities of TYA5, TYB5, and TYB1 were evaluated. HIV protease activity (the rate of enzyme reaction) was determined by use of a surface-enhanced laser desorption/ionization (SELDI)-MS method for quantitation of the parent peptide substrate (see Supporting Information).[19,20] As shown in Table 1 (K_i), the protease inhibitory activities of TYA5 and TYB5 were stronger than those of saquinavir and crixivan.[4] TYA5 and TYB5 showed almost the same protease inhibitory activity, while TYA5 showed 7-fold higher anti-HIV activity in cells than TYB5. A suitable correlation between protease inhibitory activity and anti-HIV activity for these compounds was not apparent. TYB1 also exhibited strong protease inhibitory activity, although this activity was weaker than that of

the corresponding peptidic compound TYB5. Replacement of the amide bond by an (*E*)-alkene at the P_1-P_2 position of TYB5 caused a significant decrease both in protease inhibitory activity and in anti-HIV activity. This suggested either that insertion of an EADI at the P_1-P_2 position or an increase in hydrophobicity is not suitable. Significant loss of anti-HIV activity of TYB1 might underline the importance of the P_1-P_2 amide bond for interaction with the enzyme. This might also explain the complete loss of anti-HIV activity of TYA1−4. However, purely nonpeptidic HIV protease inhibitors derived from substrate-based transition state-mimic king structures have not been reported to date. TYB1 is a novel inhibitor containing no amide bonds that possesses significant activity ($K_i = 0.38$ nM, $IC_{50} = 160$ nM). TYB1 also exhibits effective activity (a third to one-half potency) against MDR strains, except for HIV_{TM} (Table 2). Further modification of TYB1 might lead to development of potent nonpeptidic inhibitors. Of note, Rich et al. have also reported computer-assisted nonpeptidic inhibitors of aspartic peptidases.[21]

In conclusion, novel HDI-containing HIV protease inhibitors, TYA5 and TYB5, which are highly effective even against MDR strains, have been found by combining substructure units of reported inhibitors. Furthermore, a purely nonpeptidic inihibitor TYB1 has been developed based on TYB5.

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Supporting Information Available: Experimental procedures and characterization data of novel synthetic compounds, procedures of biological assays, synthetic scheme for EADI **15** (Scheme S1), X-ray crystallographic data for **27b**, and HPLC charts for TYA1, TYA2, TYA5, TYB1, TYB2, and TYB5. This material is available free of charge via the Internet at http://pubs.acs.org.

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