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A NEW CLASS OF ANTI-HIV AGENTS: SYNTHESIS AND ACTIVITY OF CONJUGATES OF HIV PROTEASE INHIBITORS WITH A REVERSE TRANSCRIPTASE INHIBITOR

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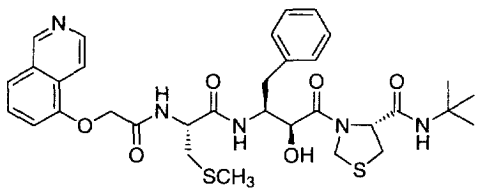
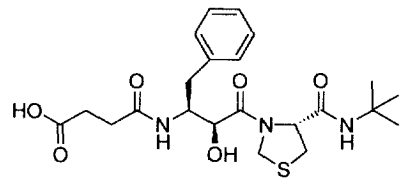
Abstract: Conjugates of HIV protease inhibitors with a reverse transcriptase inhibitor were synthesized, which expressed excellent antiviral activity compared with that of the individual components. The remarkable antiviral activity of the conjugated compounds may be due to their penetration into the cell and later splitting into two different classes of anti-HIV agents.

HIV-1 protease and reverse transcriptase are major targets for the prevention of HIV proliferation.[1] The inhibitors of these enzymes are employed for the chemotherapy of AIDS in combination.[2] We have made efforts to develop highly potent HIV protease inhibitors and found KNI-272 (Figure 1), a tripeptide inhibitor containing a transition-state mimic, (*2S, 3S*)-3-amino-2-hydroxy-4-phenylbutyric acid (allophenylnorstatine, Apns).[3,4] In the study of structure-activity relationship, we found that KNI-357 (**1a**), a dipeptide inhibitor containing a free carboxylic acid at P2, had good enzyme inhibitory activity. This type of inhibitors, **1a–5a** (Table 1), showed potent enzymatic inhibition, whereas the antiviral activities were weak.[5] Since it was supposed that the free



KNI-272



KNI-357 (**1a**)

Figure 1. Structure of tripeptide inhibitor, KNI-272 and dipeptide inhibitor, KNI-357.

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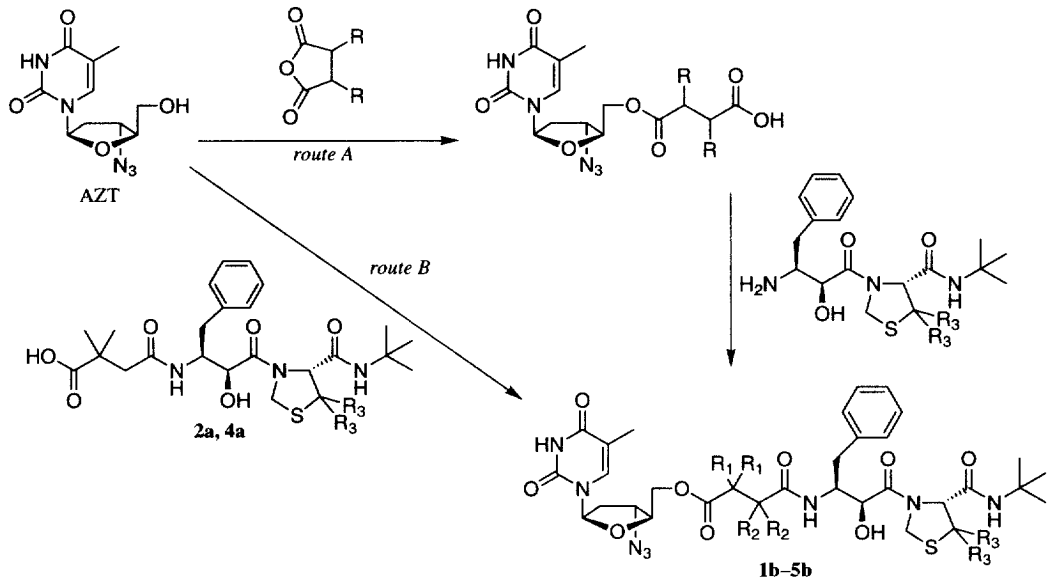


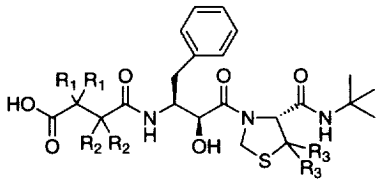
Figure 2. Synthetic route to conjugated anti-HIV agents, **1b–5b.**

carboxylic acid at P2 was not suitable for the penetration across the cell membrane, we considered that prodrug approach, conversion of a free acid into an ester, would be effective to increase the anti-HIV activity. Moreover, an adoption of a reverse transcriptase inhibitor[6] as the alcohol component was expected to enhance the anti-HIV activity. In this paper, we describe the synthesis and activity of prodrugs of the HIV protease inhibitors conjugated with a reverse transcriptase inhibitor, 3′-azido-3′-deoxythymidine (AZT).

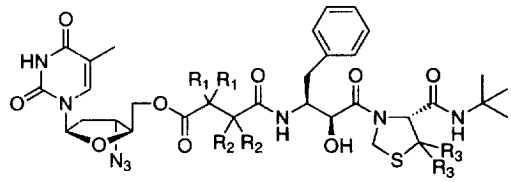
The conjugated compounds (**1b–5b**) were synthesized according to the scheme shown in Figure 2. Compounds **1b, 3b** and **5b** were prepared *via route A*. AZT was coupled with the corresponding succinyl anhydrides in the presence of dimethylaminopyridine (DMAP) (0.1 eq.) in DMF–$CHCl_3$ (1:1).[7] Condensation of the resulting half esters and the amine components using DCC–1-hydroxybenzotriazole (HOBt) method in DMF gave the desired products (58–95 %, two steps). From the consideration of regioselectivity of succinyl moieties, compounds **2b** and **4b** were prepared by coupling **2a** or **4a** with AZT by DCC–DMAP method (*via route B*).

At first, the activities of HIV-1 protease were measured in the presence of 5 μM of synthetic compounds (Table 1). The conversion into the conjugates decreased the inhibitory activity in all the cases. These results show that AZT is not suitable as P3 ligand and the hydrogen of carboxylic acid may be important for interaction with the enzyme. Next, we determined the antiviral activities of **1b–5b** using HIV-1 IIIB/CEM-SS assay system. The conjugates **1b–5b** showed remarkably potent activities, though the antiviral activities of **2a–5a** were very poor. Of those compounds, **4b** (KNI-684) showed the lowest EC_{50} (19 nM), which was 6.6 times more potent than AZT and 2.1 times than KNI-272.

Table 1. HIV protease inhibitory and antiviral activities



1a–5a



1b–5b

compound	R_1	R_2	R_3	inhibition of HIV protease[a]	EC_{50} HIV-1 IIIB/MT-4	EC_{50} HIV-1 IIIB/CEM-SS	relative potency
1a (KNI-357)	H	H	H	74 %	N.D.[b]		
2a (KNI-391)	Me	H	H	93 % (20%)	>2000 μM		
3a (KNI-547)	H, Me[c]	H, Me[c]	H	97 % (32%)	>2000 μM		
4a (KNI-413)	Me	H	Me	99 % (76%)	52 μM		
5a (KNI-549)	H, Me[c]	H, Me[c]	Me	99 % (78%)	225 μM		
1b (KNI-679)	H	H	H	14 %		42 nM	3.0
2b (KNI-680)	Me	H	H	9 %		27 nM	4.7
3b (KNI-681)	H, Me[c]	H, Me[c]	H	29 %		27 nM	4.7
4b (KNI-684)	Me	H	Me	41 %		19 nM	6.6
5b (KNI-685)	H, Me[c]	H, Me[c]	Me	69 %		90 nM	1.4
AZT	–	–	–	–		126 nM	1.0
KNI-272	–	–	–	100 %	0.57 μM	40 nM	–

[a]% of inhibition in the presence of 5 μM or 50 nM (in parenthesis) of inhibitors, [b]not determined, [c]mixture of (*2R, 3S*) and (*2S, 3R*).

From the study of structure-activity relationship of protease inhibitors, we learned that a substitution of *t*-butylamide at P2′ moiety with 2-methylbenzylamide gave a more potent inhibitor.[8] Therefore, we prepared conjugates of AZT with inhibitors containing 2-methylbenzylamide at P2′. A series of conjugated compounds, **6b–9b** (Table 2), were obtained by the same manner as above and their anti-HIV activities were determined by HIV-1 IIIB/CEM-SS assay system. As the results, the potency of **6b–9b** in the enzymatic assay were decreased compared with **6a-9a**, but the antiviral activities were potent. This is almost same as the case of **1b–5b**. In particular, **8b** (KNI-694) showed extremely high antiviral potency that is 46 times more potent than that of AZT. These results support our hypothesis, i.e. the conjugated compounds penetrate across the cell membrane and then split into the protease inhibitor and the reverse transcriptase inhibitor which act on the different targets with synergistic effect.

In conclusion, esterification of the hydroxyl group of AZT with HIV protease inhibitors containing free carboxylic acid gave remarkably potent anti-HIV agents. The antiviral activities of the conjugated compounds were considerably high compared with that of the individual components. Based on the prodrug concept as well as conjugation of two different class of anti-HIV agents, we had developed novel potent anti-HIV agents.

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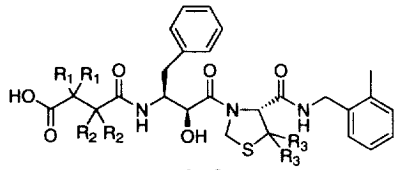
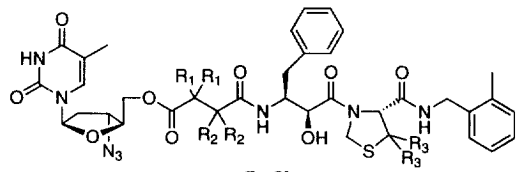
Table 2. Protease inhibitory and antiviral activities



6a–9a



6b–9b

compound	R_1	R_2	R_3	inhibition of protease[a]	EC_{50} HIV-1 IIIB /CEM-SS	relative potency
6a (KNI-689)	Me	H	H	87 %	N.D.[b]	
7a (KNI-690)	H, Me[c]	H, Me[c]	H	88 %	N.D.[b]	
8a (KNI-852)	Me	H	Me	100 % (78%)	inactive	
9a (KNI-691)	H, Me[c]	H, Me[c]	Me	100 % (76%)	N.D.[b]	
6b (KNI-692)	Me	H	H	11 %	3.8 nM	2.9
7b (KNI-693)	H, Me[c]	H, Me[c]	H	57 %	8.9 nM	1.2
8b (KNI-694)	Me	H	Me	83 %	0.24nM	46
9b (KNI-695)	H, Me[c]	H, Me[c]	Me	96 % (39%)	7.3 nM	1.5
AZT	–	–	–	–	11 nM[9]	1.0

[a]% of inhibition in the presence of 5 μM or 50 nM (in parenthesis) of inhibitors, [b]not determined, [c]mixture of (*2R, 3S*) and (*2S, 3R*).

References and Notes:

1. Helm, K. *Biol Chem.* **1996**, *377*, 765.
2. Craig, J. C.; Whittaker, L.; Duncan, I. B.; Roberts, N. *Antiviral Chemistry & Chemotherapy* **1994**, *5*, 380.
3. a) Mimoto, T.; Imai, J.; Kisanuki, S.; Enomoto, H.; Hattori, N.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1992**, *40*, 2251. b) Kageyama, S.; Mimoto, T.; Murakawa, Y.; Nomizu, M.; Ford, H. Jr; Shirasaka, T.; Gulnik, S.; Erickson, J.; Takada, K.; Hayashi, H.; Broder, S.; Kiso, Y.; Mitsuya, H. *Antimicrob. Agent. Chemother.* **1993**, *37*, 810.
4. Kiso, Y. *Biopolymers* **1996**, *40*, 235-244.
5. a) Mitoguchi, T.; Nakata, S.; Enomoto, H.; Kimura, T.; Akaji, K.; Hattori, N.; Kato, R.; Mimoto, T.; Kiso, Y. In: *Peptide Chemistry 1995*; Nishi, N., Ed.; Protein Res. Found.: Osaka, 1996; pp. 373–376. b) Kiso, Y.; Yamaguchi, S.; Matsumoto, H.; Mimoto, T.; Kato, R.; Nojima, S.; Takaku, H.; Fukazawa, T.; Kimura, T.; Akaji, K. *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 87.
6. a) Mitsuya, H.; Weinhold, K.; Furman, P. A.; Clair, M. H. S.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. USA.* **1985**, *82*, 7096. b) Camplo, M.; Niddam, V.; Barthélémy, P.; Faury, P.; Mourier, N.; Simon, V.; Charvet, A. S.; Trabaud, C.; Graciet, J. C.; Chermann, J. C.; Kraus, J. L. *Eur. J. Med. Chem.* **1995**, *30*, 789.
7. Tadayoni, B. M.,; Friden, B. M.; Walus, L. R.; Musso, G. F. *Bioconjugate Chem.* **1993**, *4*, 139.
8. Mimoto, T.; Kato, R.; Takaku, H.; Misawa, S.; Fukazawa, T.; Nojima, S.; Terashima, K.; Satoh, H.; Shintani, M.; Kiso, Y.; Hayashi, H. In: Proceedings of the 1st International Peptide Symposium, in press.
9. The EC_{50} value of AZT in this table varied comparing with the experiment of Table 1 carried out at different time. The difference of the values of EC_{50} for AZT may be caused by the different population of the spontaneous variant in wild type CEM cell line used as mentioned by Törnevik et al.[10]
10. Törnevik, Y.; Ullman, B.; Balzarini, J.; Wahren, B.; Eriksson, S. *Biochem. Pharmacol.* **1995**, *49*, 829.