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Effect of the Acyl Groups on *O*→*N* Acyl Migration in the Water-Soluble Prodrugs of HIV-1 Protease Inhibitor

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Abstract—To improve the low water-solubility of HIV-1 protease inhibitors KNI-272, -279 and -727, we previously reported the water-soluble prodrugs of these inhibitors based on *O*→*N* intramolecular acyl migration reaction. These prodrugs were rapidly converted to the corresponding parent drugs under physiological conditions. To understand the steric and electrostatic effects of *O*-acyl moiety on the migration rate, we examined several types of prodrug. A remarkably slow migration was observed in the benzoyl-type prodrugs, and Hammett plot of migration rate constants of *p*-substituted benzoyl-type prodrugs gave a linear free energy relationship.

Introduction

Since HIV-1 protease (HIV-1 PR) recognizes hydrophobic sequences in viral precursor proteins,[1-3] most HIV-1 protease inhibitors, which were developed based on the substrate structures, showed low water-solubility leading to low gastrointestinal absorption.[4,5] To solve this problem, we recently developed water-soluble prodrugs[6,7] of potent tripeptide-type HIV-1 PR inhibitors, KNI-272 (**2a**) and-279 (**2b**)[8] and dipeptide-type inhibitor, KNI-727 (**4**),[6c,9] using the *O*→*N* intramolecular acyl migration reaction (Fig. 1).[10] These prodrugs, that is the *O*-acyl isoforms of the parent inhibitors, can increase water-solubility by the hydrophilic effect of a newly present ionized amino group. More than 4000-fold higher water-solubility was obtained in comparison with parent compounds. These prodrugs were stable in the solid state as an HCl salt and in an acidic solution (pH 2.0), which corresponded to the pH of gastric juice, but rapidly converted to the parent compounds under physiological conditions. These prodrugs have amino acid-type or phenoxyacetyl-type *O*-acyl groups. On the other hand, an important compound, second generation HIV-1 PR inhibitor KNI-764 (JE-2147)[9a] has a benzoyl-type structure. Since a range of migration rates is necessary for higher gastrointestinal absorption, one of our goals is to understand the structural effects of acyl groups on the migration rate. Hence, in the present study, to clarify the steric and electrostatic effects of *O*-acyl moiety on the migration rate, we evaluated phenoxyacetyl-type, phenylacetyl-type and benzoyl-type prodrugs.

Chemistry

The synthesis of prodrug **5**–**11** is shown in Scheme 1A. The starting compound **12**[6d,8a,9a] was coupled with **13a**–**g** using DCC in the presence of a catalytic amount of DMAP to afford Boc-protected prodrugs. The Boc group was removed with 4 N HCl/dioxane to afford the HCl salts of prodrugs **5**–**11**. The synthesis of parent compounds is shown in Scheme 1B. **14**[8a,9a] and **13a**–**g** were coupled using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (EDC·HCl) in the presence of HOBt (*N*-hydroxybenzotriazole) to afford parent compounds **15**–**21**.

Results and Discussion

Effect of the phenyl ring on *O*→*N* acyl migration

A previous study using tripeptide-type inhibitors, KNI-272 and -279, revealed that steric hindrance at the

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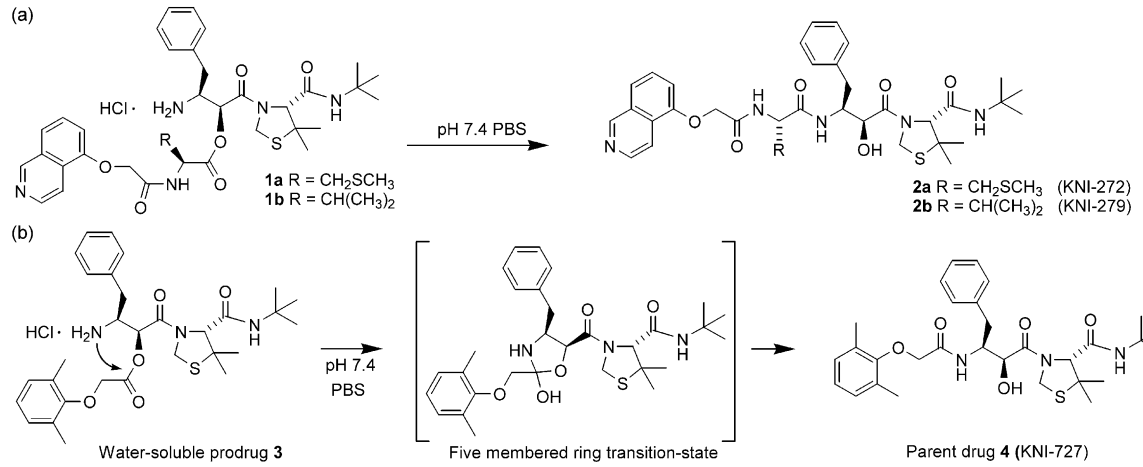
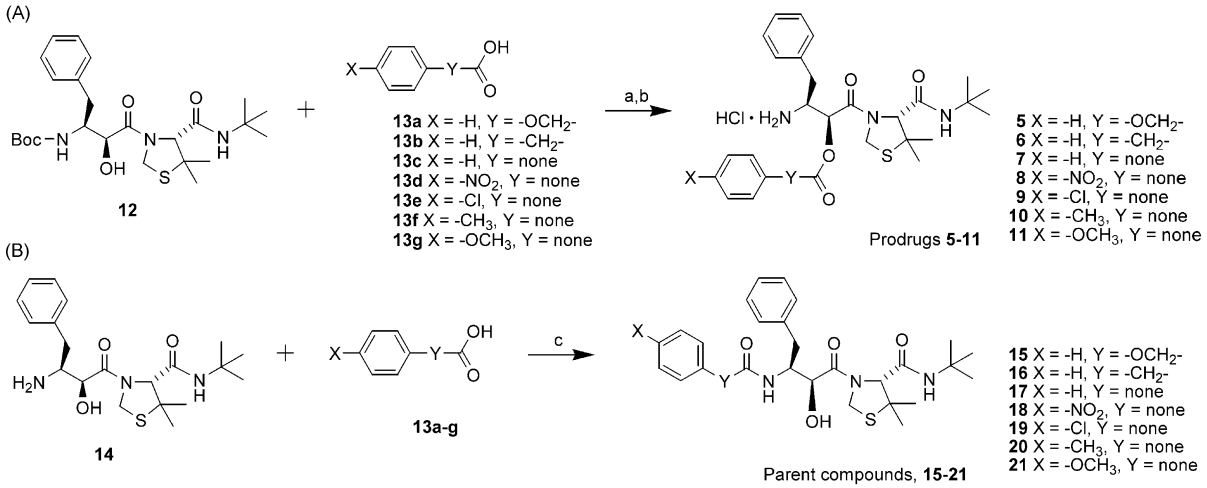


Figure 1. Prodrugs based on *O*→*N* intramolecular acyl migration reaction: (a) prodrugs (**1a**, **1b**) of KNI-272 and -279; (b) prodrug (**3**) of KNI-727.



Scheme 1. Synthesis of prodrugs **5–11** (A) and parent drugs **15–21** (B). Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, rt; (b) 4 N-HCl/dioxane, rt; (c) EDC·HCl, HOBt, DMF, rt.

α-position of the *O*-acyl moiety decreased the migration rate of the prodrugs.[6d] Here, we have focused on dipeptide-type inhibitors in which the structures of acyl moiety are the relatively simple carboxylic acid, with a phenyl group such as phenoxyacetic or benzoic acid derivatives, for a precise structure-migration rate relationship study. Firstly, the migration rates of prodrugs **5–7** were determined to understand the effect of the position of the phenyl ring. Prodrugs **5–7** were dissolved in phosphate buffered saline (PBS, pH 7.4) and incubated at 37 °C, then *O*→*N* acyl migration was monitored by HPLC.[6d] Figure 2 and Table 1 show the time course of migration, and the $t_{1/2}$ values and rate constants in **5–7**, respectively. The rate constants were calculated using the fitting eq 1.[11–14]

$$[A]_t = A_{MAX} \times (1\text{-Exp}(-k \times t)) \quad (1)$$

[t, incubation time; k, rate constant of migration; A_{MAX}, maximum concentration of the parent compound (initial concentration of prodrug); [A], concentration of the parent compound].

Prodrug **5,** having the phenoxyacetyl group ($pK_a = 3.2$),[15] showed the fastest migration rate, which was a similar rate to prodrug **3** having the 2,6-dimethylphenoxyacetyl group, suggesting that the 2,6-dimethyl group in the phenyl ring did not affect the migration rate. In prodrug **6**, a slightly slower migration was observed. This was probably due to the weaker electron-withdrawing effect of **6** ($pK_a = 4.31$). However, a remarkably slow migration was observed in prodrug **7**. Since benzoic acid in **7** has a similar pK_a value ($pK_a = 4.20$) to phenylacetic acid in **6**, the observed slower migration was probably due to the steric effect of the phenyl ring, suggesting that the steric effect of the phenyl ring significantly affects the *O*→*N* acyl migration rate only in the cases where the phenyl ring was directly connected to the carbonyl carbon.

Electrostatic effect of the substituted benzoyl groups on the *O*→*N* acyl migration rate

Because the observed slow migration rate of the benzoyl group was appropriate for considering the electrostatic effect on the migration rate, and the highly potent inhibitor, KNI-764,[9a] which has been under clinical studies,

possesses a 3-hydroxy-2-methylbenzoyl group as the acyl moiety, we next determined the $t_{1/2}$ values and the rate constants of prodrugs **8**–**11** with *p*-substituted benzoyl groups using the same manner mentioned above. The time course of migration of these prodrugs is shown in Figure 3a. By introduction of an electron-withdrawing function, such as a nitro (**8**) group or a chlorine atom (**9**), an accelerated migration rate was observed, while by introduction of an electron-donating group, such as methyl (**10**) or methoxy (**11**) groups, the migration rate was decelerated. To analyze these results, the $\log(k_X/k_H)$ values of **7**–**11** were calculated using the Hammett eq 2.[16]

$$\log(k_X/k_H) = \rho\log(K_X/K_H) = \rho\sigma_X \quad (2)$$

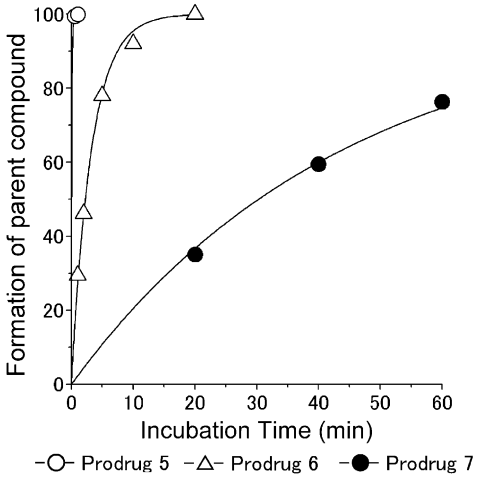


Figure 2. Formation of parent compounds from prodrug **5**–**7** in pH 7.4 PBS at 37 °C.

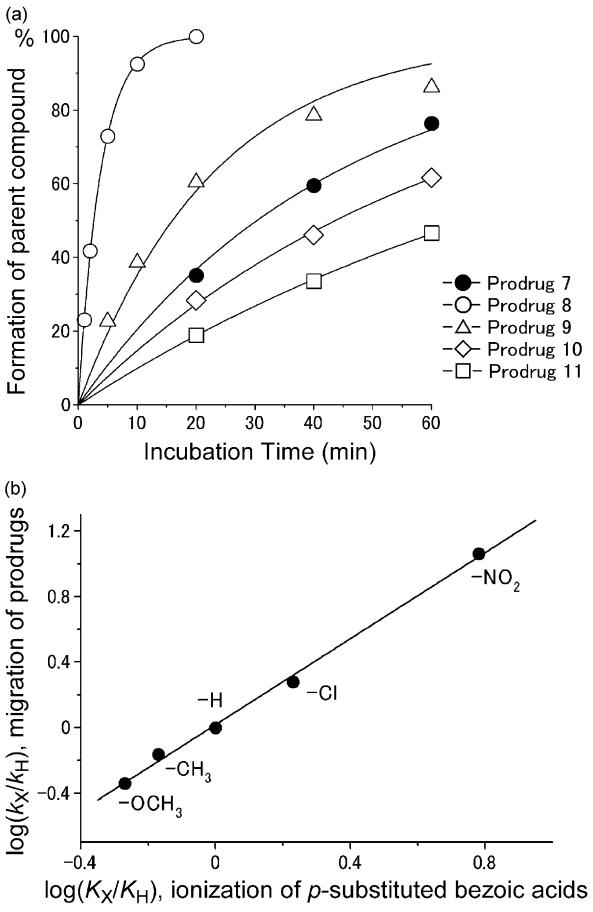
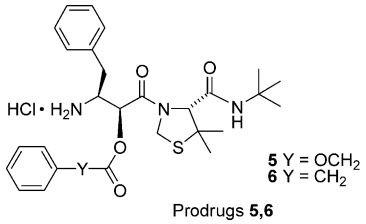
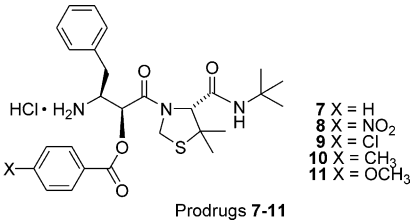


Figure 3. (a) Formation of parent compounds from prodrugs **7**–**11** in pH 7.4 PBS at 37 °C; (b) the correlation of migration rate constants of prodrugs against acid dissociation constants of *p*-substituted benzoic acids.

Table 1. Water-solubility and *O*→*N* acyl migration rate of prodrugs **5**–**11**



Prodrugs **5,6**



Prodrugs **7-11**

Prodrug	Solubility[a] (mg/mL)		Ratio of solubility (prodrug / parent drug)	$t_{1/2}$ (min)[b]	Rate constant[c] k (s^{-1})
	Prodrug	Parent drug			
5	21	0.047	449	<1[d]	$>115\times10^{-4}$
6	33	0.097	340	2.2	52.5×10^{-4}
7	15	0.120	125	30	3.81×10^{-4}
8	13	0.057	228	2.6	44.0×10^{-4}
9	4.3	0.028	154	16	7.24×10^{-4}
10	6.2	0.040	155	44	2.65×10^{-4}
11	16	0.076	211	67	1.73×10^{-4}
3 (prodrug of KNI-727)[e]	13	0.0015	8666	<1[d]	$>115\times10^{-4}$

[a]Solubility in pure water at 25 °C.
[b]$t_{1/2}$ is the time required for 50% release of parent drugs in PBS (pH 7.4) at 37 °C.
[c]Migration rate constant (k) of prodrugs in pH 7.4 PBS at 37 °C. The k-values were calculated using eq 1; $[A]_t = A_{MAX}\times(1-\mathrm{Exp}(-k\times t))$.
[d]Prodrugs **5** and **3** showed the similar $t_{1/2}$ values (3.2 and 4.5 min) in PBS (pH 5.5) at 37 °C.
[e]See ref 6c.

(ρ, reaction constant; σ_X, substituent constant; k, rate constants of migration of prodrugs, K, acid dissociation constants of *p*-substituted benzoic acids at 25 °C).

Acid dissociation constants of *p*-substituted benzoic acids in water at 25 °C were used as a standard reference reaction, and the migration rate constants of prodrugs **7–11** were plotted. As shown in Figure 3b, a linear free energy relationship ($\rho = 1.31$) was obtained. This suggests that the migration reaction proceeds under a single mechanism or the same rate-limiting step,[16] and the migration rate of these prodrugs depends only on the electrostatic effect under a constant steric effect. This is probably due to the *p*-substitution not changing the steric effect at the carbonyl part. Thus, the slope of obtained Hammett correlation can be valuable to predict the migration rates of benzoyl-type prodrugs containing *p*-substitution. However, in the case of *m*- or *o*-substitution, varied steric effects should also be considered for the prediction of the actual migration rate.

Water-solubility of the prodrugs

Water-solubility of the prodrugs was determined by HPLC analysis,[6d] and compared to that of their parent compounds (Table 1). An excessive amount of prodrug and parent compound was suspended in pure water under sonification for 5 min at 25 °C, and filtered by a centrifugal membrane filter of 0.45 μm pore size. Water solubility was calculated from each peak area by HPLC, and compared with the standard solution. In every case, prodrugs exhibited more than 100-fold higher water-solubility than parent compounds.

Conclusion

We synthesized water-soluble prodrugs **5–11** with a phenyl group at the acyl moiety to understand the structural factors affecting the $O \rightarrow N$ acyl migration rate of the prodrugs. The steric effect of the phenyl group was predominantly affected the migration when it was directly connected to the carbonyl carbon (i.e., benzoyl group). In the *p*-substituted benzoyl-type prodrugs, Hammett plot of migration rate constants gave a linear free energy relationship, and the $O \rightarrow N$ acyl migration rate was predicted precisely. These findings may contribute to the efficient design of prodrugs based on $O \rightarrow N$ acyl migration reaction required for providing an expected appropriate gastrointestinal absorption.

Acknowledgements

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