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IRREVERSIBLE INHIBITIONS OF SERINE PROTEASES BY PEPTIDYL ALLYLIC HALIDE DERIVATIVES

Tsuyoshi Ohba, Eitatsu Ikeda, Jun Wakayama and Hisashi Takei*

Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology Nagatsuta 4259, Midoriku, Yokohama 226, Japan

Abstract. Peptidyl 4-amino-5-phenyl-2-pentenyl bromide (7a, 9a, 10a) and chloride derivatives (7b, 9b, 10b) were found to be active-site directed irreversible inhibitors of α -chymotrypsin but did not show any irreversible inhibitory activity toward porcine pancreatic elastase.

Serine proteases are concerned in numerous reactions *in vivo* and, therefore, selective and irreversible inhibitors are useful tools in the study of each serine protease mechanism and in the design of new inhibitors as drugs.^{1,2,3}

Although a number of irreversible inhibitors containing electrophilic moiety in their structures such as peptidyl chloromethyl ketones^{4,5,6}, peptidylphosphonates^{7,8}, and alkyl isocyanates^{9,10} have been reported, there is no report about the potency and the selectivity of allylic halide derivatives as an irreversible inhibitor of serine proteases, to the best of our knowledge. Since it has been known that amide bond resembles to carbon-carbon double bond in three dimentional structure^{11,12}, we designed peptidyl allylic halide derivatives as novel irreversible inhibitors of serine proteases.



Peptidyl allylic halide derivatives would be thought to show high affinity toward serine proteases and react with active-site nucleophiles. Although allylic fluorides are not so electrophilic species as allylic bromides or chlorides, we assumed that the hydrogen bonding between fluorine atom and amide proton in active-site would assist the displacement of fluorine atom^{13,14}. We chose α -chymotrypsin, which favors peptide substrates possessing Trp, Tyr, and Phe side chains at position P₁, as our target enzyme and

synthesized a series of peptidyl 4-amino-5-phenyl-2-pentenyl halide derivatives 7a-b, 9a-b, 10a-b, 12a-c considering the substrate specificity of α -chymotrypsin.





(a) EDC•HCl, HOBt, HCl•HN(OMe)Me, CH₂Cl₂ (92 %); (b) LiAlH₄, THF, 0 °C (95 %);
(c) (EtO)₂POCH₂CO₂Et, NaH, THF, 0 °C (75 %); (d) DIBAL-H, BF₃•OEt₂, CH₂Cl₂ (80 %);
(e) 1) 4 N-HCl/dioxane (quant.); 2) Ac₂O, NEt₃, CH₂Cl₂ (quant.); (f) 1) K₂CO₃aq., MeOH (quant.);
2) 7a: PPh₃, CBr₄, CH₂Cl₂ 0 °C (24 %)/7b: PPh₃, CCl₄, THF, reflux (30 %)

Scheme 2



(a) 1) 4 N-HCl/dioxane (quant.), 2) Z-(Gly)_n-OH, EDC-HCl, HOBt, CH₂Cl₂ (8a: 59 %, 8b: 76 %); (b) 9a, 10a: PPh₃, CBr₄, CH₂Cl₂ (9a: 36 %, 10a: 45 %) / 9b, 10b: PPh₃, CCl₄, THF, reflux (9b: 51 %, 10b: 39 %)



(a) Et_2NSF_3 , DME, -78 °C (37 %); (b) 1) 4 N-HCl/dioxane (quant.), 2) 12a: Ac₂O, Pyridine (76 %); 12b: Z-Gly-OH, EDC • HCl, DMAP, CH₂Cl₂-dioxane (91 %); 12c: Z-Gly-Gly-OH, EDC•HCl, DMAP, CH₂Cl₂-dioxane (68 %)

All compounds were synthesized using known procedures via optically active common intermediate 5^{15} , which was synthesized from compound 4 by the method of Morikawa et al.^{16,17} as shown in Scheme 1¹⁸. A series of peptidyl allylic fluorides were synthesized as shown in Scheme 3. Fluorination of compound 5 was carried out with diethylaminosulfur trifluoride (DAST) in 1,2-dimethoxyethane¹⁹.

First, we evaluated the inhibitory activity of a series of bromides **7a**, **9a**, **10a** and obtained the secondorder rate constants (kobsd /[I]) according to the literature procedures ^{8,20}. Incubation of compound **7a** with α -chymotrypsin resulted in a time-dependent loss of enzyme activity with kobsd /[I] = 14 M⁻¹s⁻¹ ([I] = 101 μ M). The inactivation assay of α -chymotrypsin by compound **7a** (101 μ M) in the presence of the substrate (Suc-Ala-Ala-Pro-Phe-NA, 0.5 mM and 0.25 mM) resulted in a decrease in the inactivation rate (kobsd /[I] = 3.5 M⁻¹s⁻¹ and 5.0 M⁻¹s⁻¹, respectively) and dialysis of assay solution against buffer solution (pH 7.5, 24 h) at 4 °C did not restore any enzyme activity, indicating that compound **7a** is active-site directed. Furthermore, dipeptidyl and tripeptidyl derivatives **9a**, **10a** exhibited more potent inhibitory activity with kobsd /[I] = 215 M⁻¹s⁻¹ ([I] = 27 μ M) and 1238 M⁻¹s⁻¹ ([I] = 7 μ M), respectively. The inactivation was selective because the bromides exhibited no inhibitory activity toward porcine pancreatic elastase (PPE). However, it was found that the bromides decompose gradually under the assay conditions.

Next, we investigated a series of chlorides 7b, 9b and 10b. The chlorides are stable under the assay conditions and exhibited irreversible inhibitory activity toward α -chymotrypsin similarly to the bromides. Mono amino acid derivative 7b exhibited rather weak inhibitory activity with $k_{obsd}/[I] = 0.8 \text{ M}^{-1}\text{s}^{-1}$ ([I] = 476 μ M), compared to corresponding the bromide derivative 7a, and time-dependent loss of enzyme activity was not observed at low concentration (< 200 μ M). However, dipeptidyl and tripeptidyl derivatives 9b, 10b exhibited significant improvement on the inactivation rate at low inhibitor concentrations with $k_{obsd}/[I]$ value = $12 \text{ M}^{-1}\text{s}^{-1}$ ([I] = 28 μ M) and 61 M⁻¹s⁻¹ ([I] = 17 μ M), respectively. In addition, the inactivation was selective because both dipeptidyl and tripeptidyl chlorides exhibited no irreversible inhibitory activity toward PPE. These data indicates that the chemical reactivity of the chlorides is modest but the inhibitory activity of the chlorides can be improved by increasing the affinity toward enzyme. Therefore, the chlorides would be also selective and potent irreversible inhibitors of serine proteases as well as the bromides.

On the other hand, a series of peptidyl allylic fluorides exhibited no irreversible inhibitory activity toward α -chymotrypsin. Therefore, contrary to our speculation, the effect of the hydrogen bonding seems not to be expected for the displacement of fluorine atom in this case.

	···· ··· ··· ··· ···	enzyme			
-		α-Chymotrypsin ^a		b PP elastase	
inhibitor	Hal	[I] ; inhibitor concentration (μM)	<i>kobsd /</i> [I] (M ⁻¹ s ⁻¹)	[I] ; inhibitor concentration (µM)	kobsd /[I] (M ⁻¹ s ⁻¹)
7a	Br	101	14	101	N. I. ^C
9a	Br	25	215	25	N. I.
10a	Br	7	1238	15	N. I.
7b	Cl	476	0.8		—
9b	Cl	28	12	600	N. I.
10b	Cl	17	61	97	N. I.
12a	F	13.5 mM	N. I.	—	
12b	F	1.05 mM	N. I.		
12c	F	4.4 mM	N. I.		—

Table 1. Inactivation of Serine Proteases by Peptidyl Allylic Halide Derivatives

^a α -Chymotrypsin (1.6 μ M) was incubated in 500 μ l of buffer (0.1 M sodium phosphate buffer, 0.5 M NaCl, 5 % Me₂SO, pH 7.8 at 25 °C) containing inhibitors. At various time intervals, 10 μ l aliquots were withdrawned and assayed with 1500 μ l of Suc-Ala-Ala-Pro-Phe-NA (0.5 mM, buffered as above) as a substrate (Suc=succinyl; NA=4-nitroanilide); ^bPP elastase (11 μ M) was incubated in 500 μ l of above buffer containing inhibitors. At various time intervals, 50 μ l aliquots were withdrawned and assayed with 1950 μ l of Suc-Ala-Ala-NA (0.7 mM, buffered as above) as a substrate; ^cno inactivation

In summary, a series of peptidyl 4-amino-5-phenyl-2-pentenyl bromide and chloride derivatives has been found to be selective irreversible inhibitors of α -chymotrypsin. From the kinetic data, the chlorides would be regarded as not classic affinity labels but quiescent affinity labels such as peptidyl acyloxymethylketones^{21,22},

specific inhibitors of cysteine proteases. Since the inactivation rates of both the bromides and the chlorides were improved significantly by introducing simple peptide chain (such as Z-Gly- or Z-Gly-Gly-) into the amino group of mono amino acid derivatives, our compounds will be more potent inhibitors by introducing more suitable peptide chain for target enzyme. In addition, optically active peptidyl allylic bromides and chlorides can be prepared from α -amino acid without difficult procedures and, therefore, they would be useful irreversible inhibitors for the future enzyme studies.

Two inactivation mechanisms have been postulated as shown in Scheme 4. One is the reaction with Ser 195 by S_N2' fashion and the other with His 57 by S_N2 fashion. Studies leading to the proof of the precise mechanism and the extention of our design to other serine proteases are now in progress.

Scheme 4. Postulated Inactivation Mechanism by Peptidyl Allylic Bromides and Chlorides



References and Notes

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- Abbreviations: Ac, acetyl ; Boc, *tert*.-butyloxycarbonyl ; DIBAL-H, diisobutylaluminium hydride ; DMAP, 4-dimethylaminopyridine ; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxybenzotriazole ; NA, 4-nitroanilide ; Suc, succinyl ; THF, tetrahydrofuran ; Z, benzyloxycarbonyl
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