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"INVERSE" TYPE OF SYNTHETIC INHIBITORS OF TRYPSIN, S-ω-AMINOALKYL THIOESTERS

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The inhibitory effects on trypsin of a series of ω-aminoalkyl thiobenzoates are explored in comparison with ω-aminoalkyl benzoates. Among the thioesters evaluated, S-4-aminobutyl and S-5-aminopentyl thiobenzoates are new "inverse" types of the most promising reversible inhibitors in inhibitory order, comparable to benzamidine.

KEYWORDS —— trypsin inhibitor; ω-aminoalkyl thiobenzoate; inverse type inhibitor

There have been extensively explored highly effective inhibitors of proteases such as trypsin, plasmin, kallikrein and thrombin, which would serve well as diagnostic tools in a wide range of enzyme research and for medical use. Among the simple synthetic inhibitors developed so far, amidino and guanidino-carboxylic acid derivatives greatly inhibit certain proteases in reversible or irreversible manner, and some are of clinical importance.[1)]

On the other hand, Kanaoka introduced unique compounds such as p-amidinophenyl esters as "inverse" types of the substrates of trypsin. These are of great value for structural and mechanistic elucidation of the trypsin-like enzymes.[2)]

In the course of the study on synthetic utility of readily available hydrolases, we have found that the "inverse" type of S-ω-aminoalkyl thioesters (1) with an appropriate methylene length have the strong inhibitory effects on trypsin. In contrast, ω-aminoalkyl benzoates and S-phenyl ω-aminothioalkanoate were much poorer inhibitors as presented partly in Table I and the Figures. This "inverse" type of thio-inhibi-

$$\text{Boc-NH-(CH}_2\text{)}_N\text{-OH} + \text{RCOSH} \longrightarrow \underset{(1)\ :\ (N=2\sim 8)}{\text{NH}_2\text{-(CH}_2\text{)}_N\text{-S-COR}}$$

tors, to our best knowledge, has no precedent in enzymology, although amino acid and peptide thioesters have been utilized as substrates for various proteases including trypsin.[3)] This paper describes the inhibitory activities of such thioesters as a new type of effective inhibitor of trypsin.

A series of thioesters evaluated in this study was prepared with the Mitsunobu reagent (azodicarboxylate and triphenylphosphine)[4)] from N-Boc-ω-amino-alcohols,[5)] in which two functional groups were linked by a two to eight methylene chain, and thiobenzoic acid, followed by amino-deprotection with hydrochloric acid in ethyl acetate. Thus, the amino thioester hydrochlorides were readily obtained as colorless crystals in 50-70% overall yields and fully characterized by spectral (IR, NMR, MS) and elemental analyses.

Table 1. Inhibitory Effects of ω-Aminoalkyl Thioesters and Reference Compounds on Trypsin-Catalyzed Hydrolysis of BAA[a] and BAPA[b]

Inhibitor	mp (°C) (HCl salt)	Inhibitor concentration for 50% inhibition (M)[a]	Ki[b]
NH_2-$(CH_2)_2$-SCOPh (2)	173	$>> 2.0 \times 10^{-3}$ (12%)[c]	—[d]
NH_2-$(CH_2)_3$-SCOPh (3)	125	4.5×10^{-4}	$> 1 \times 10^{-3}$
NH_2-$(CH_2)_4$-SCOPh (4)	106	1.5×10^{-5}	3.6×10^{-5}
NH_2-$(CH_2)_5$-SCOPh (5)	111	4.2×10^{-5}	1.2×10^{-4}
NH_2-$(CH_2)_6$-SCOPh (6)	108	2.1×10^{-4}	5.2×10^{-4}
NH_2-$(CH_2)_8$-SCOPh (7)	112	$>> 2.5 \times 10^{-4}$ (20%)[c]	—[d]
NH_2-$(CH_2)_4$-OCOPh (8)	105	$>> 2.0 \times 10^{-3}$ (16%)[c]	—[d]
NH_2-$(CH_2)_5$-COSPh (9)[e]	74	2.7×10^{-4}	—[d]
Benzamidine	168	3.0×10^{-5}	3.8×10^{-5}

a) Trypsin (Sigma, type III) solution (4.3×10^{-5} M)(in 0.001M HCl and 0.02M $CaCl_2$) (100 μL) was added to the phosphate buffer solution (pH 6.6) (10 mL) containing N-benzoyl-L-argininamide (BAA) (1.0 mM) and inhibitor (0.04 to 2.0 mM). After incubation at 30°C for 1 h , the reaction was quenched with 4N-trichloroacetic acid (100 μL). The N-benzoyl-arginine generated was determined by reversed phase chromatography (Partisil-5 ODS-3, CH_3CN/H_2O (1:9), UV-245 nm). b) N-Benzoyl-DL-arginine-p-nitroanilide (BAPA) solution was added to the preincubated (5 min) solution of trypsin and inhibitor in phosphate-buffer (pH 6.6) containing 10% DMF as co-solvent and the hydrolysis was monitored spectroscopically at 410 nm. The Ki values were determined as described by Dixon.[6] c) Percent inhibition at the concentrations specified. d) Not determined. e) Slightly decomposed non-enzymatically in buffer solutions at pH 6.6.

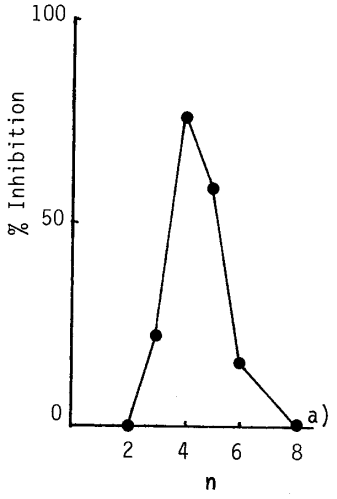

Fig. 1. Inhibitory Effects of Chain-Length of NH_2-$(CH_2)_n$-SCOPh on Tryptic Hydrolysis of BAA.

Hydrolysis was performed in phosphate-buffer (at pH 6.6 and 30°C for 1 h) at substrate and inhibitor concentrations of 1.0 mM and 0.04 mM, respectively. a) Measured in 5% DMF-buffer solution.

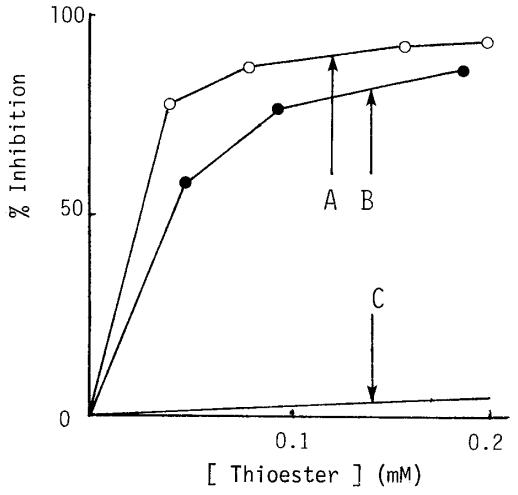

Fig. 2. Inhibition of Tryptic Hydrolysis of BAA by Thioesters (A,B) and Ester (C).

A: S-4-aminobutyl thiobenzoate (4).

B: S-5-aminopentyl thiobenzoate (5).

C: 4-aminobutyl benzoate (8).

The procedures are same as in Fig. 1 except the inhibitor concentrations.

These compounds were chemically stable enough to permit determination of the inhibitory effects on trypsin in the following assay systems. The rates of tryptic hydrolysis of N-benzoyl-L-argininamide (BAA) and N-benzoyl-DL-arginine-p-nitroanilide (BAPA) were measured in phosphate buffer solutions at pH 6.6 in the presence and absence of inhibitors. The percent inhibition was obtained by using liquid chromatography to determine the N-benzoylarginine generated during tryptic hydrolysis of BAA as a substrate at the concentration of 1.0 mM. The Ki-values were determined at various concentrations of BAPA by monitoring p-nitroaniline spectroscopically, according to the published method.[6]

In Table I are summarized the inhibitory effects of a series of S-ω-aminoalkyl thiobenzoates on the amidolytic activities of trypsin in terms of the concentrations for 50% inhibition and the Ki-values. The inhibitory effects were heavily dependent on the intramolecular distance between the two functional groups, amino and carbonyl, as indicated in Fig. 1. Among the thioesters examined, the most effective inhibitor was S-4-aminobutyl thiobenzoate (4); S-5-aminopentyl thioester (5) was the next stronger. These compounds were competitive inhibitors,[7] indicating the importance of the four to five-methylene spacers which may be highly fittable to an active site of the enzyme. In contrast, inverse ester 8 (4-aminobutyl benzoate) and S-phenyl amino-thioester (9) were very poor inhibitors, compared with the inverse type of thioesters (Fig. 2 and Table I), suggesting that the "inverse" structure of thioesters plays an important role in inhibiting trypsin amidolytic activity. Contrary to our expectations, only weak inhibition was observed with S-trans-4-(aminomethyl)-cyclohexylmethyl thiobenzoate of the preferably fixed conformation.

Inverse thio-inhibitors (4 and 5) described here are readily accessible and the structural modifications such as introduction of chirality to the polymethylene moiety are feasible. Thus, the present study represents a cornerstone toward designing specific and sensitive inhibitors for the trypsin-like enzymes of biological interest.

REFERENCES AND NOTES

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