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Design and Synthesis of Peptide-Based Carboxylic Acid-Containing Transition-State Inhibitors of Human Neutrophil Elastase

Fuminori Sato,[a,*] Yasunao Inoue,[a] Tomoki Omodani,[a] Kiyomi Imano,[b] Hiroshi Okazaki,[b] Tadashi Takemura[b] and Masanobu Komiya[b]

[a]*Department of Chemistry II, Discovery Research Laboratories, Dainippon Pharmaceutical Co., Ltd. Enoki 33-94, Suita, Osaka 564-0053, Japan*

[b]*Department of Pharmacology III, Discovery Research Laboratories, Dainippon Pharmaceutical Co., Ltd. Enoki 33-94, Suita, Osaka 564-0053, Japan*

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Abstract—In our search for a new agent, human neutrophil elastase (HNE) inhibitor, for the treatment of acute respiratory failure, we rationally designed and synthesized a series of peptide-based carboxylic acid-containing transition-state inhibitors. The presence of valyl moiety is found to be essential for potent in vitro inhibitory activity and also prevention of an undesirable toxicity. Of these, compound **9m** has the most potent in vivo effect on HNE-induced lung hemorrhage in hamsters.

Human neutrophil elastase (HNE, EC 3.4.21.37), a neutral serine protease with broad substrate specificity, is a major constituent in the azurophilic granules of human neutrophils. Under normal conditions, the proteolytic activity of the HNE is controlled by some endogenous inhibitors such as α_1-protease inhibitor, α_2-macrogloblin, secretroy leukocyte protease inhibitor, and so on. An imbalance between the HNE and endogenous inhibitors has been suggested as a major causative factor in the acute respiratory distress syndrome or the acute lung injury, which is a severe life-threatening sequel to sepsis and other conditions.[1] It is characterized by massive infiltration of neutrophils into the lungs and other organs, where the neutrophils cause tissue injury by releasing the HNE and some toxic substances. Although numerous HNE inhibitors including peptidic and nonpeptidic inhibitors have been investigated, no agent for clinical use has so far been developed.[2] For the treatment of the acute destructive diseases, the administration of a drug by injection is preferred. Therefore, the high solubility and stability in aqueous solution are essential physio-chemical properties for the inhibitor. During our search for a new agent for the treatment of such acute disorders, we have reviewed HNE inhibitors reported previously.[3] The known compounds are classified into two types based on their inhibitory mechanism: (1) acyl-enzyme inhibitor and (2) transition-state inhibitor (Fig. 1). In general, the in vitro inhibitory activities

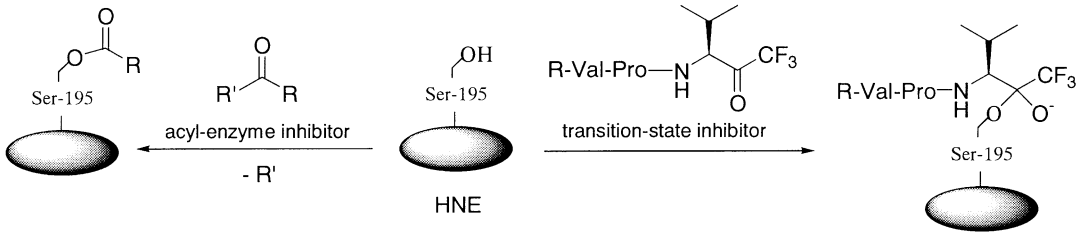

Figure 1. Inhibitory mechanisms of acyl-enzyme inhibitor and transition-state inhibitor.

*Corresponding author. Tel.: +81-6-6337-7040; fax: +81-6-6338-7656; e-mail: fuminori-sato@dainippon-pharm.co.jp

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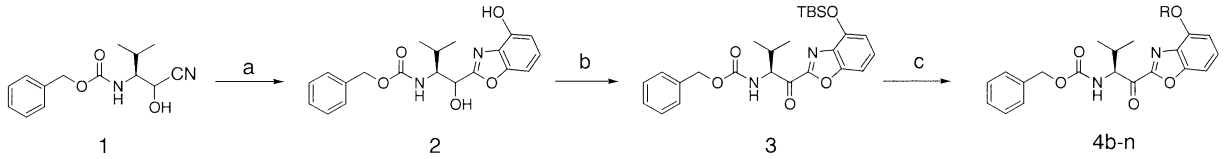


Scheme 1. (a) (i) HCl, EtOH, $CHCl_3$; (ii) 2,6-dihydroxyaniline, Et_3N, EtOH; (b) (i) TBSCl, DMAP, Et_3N, CH_2Cl_2; (ii) DMP, CH_2Cl_2; (c) (i) TBAF, THF; (ii) R-Br or R-I, NaH, DMF or RCOCl, Et_3N, CH_2Cl_2.

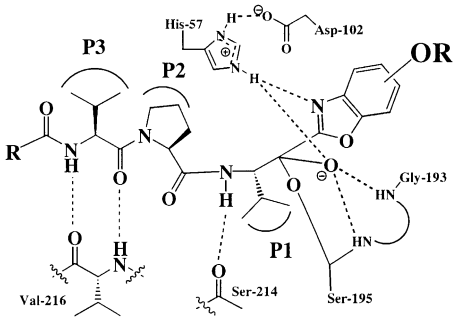
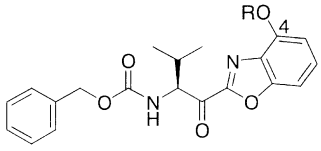


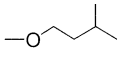
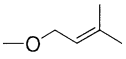
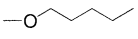
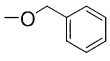
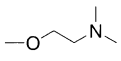
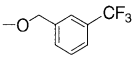
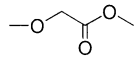
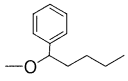
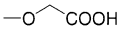
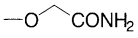
Figure 2. The inhibitory interaction between a peptidyl α-keto-benzoxazole and porcine pancreatic elastase.[4]

of the acyl-enzyme inhibitors are relatively high. However, it would be difficult to develop them to a water stable form with a low toxicity, due to their high reactivity toward nucleophiles. On the other hand, the transition-state inhibitors, such as peptide-based trifluoromethyl ketone, reversibly react with the active-site serine-195 of HNE to form a tetrahedral configuration. The carbonyl moiety (–$COCF_3$) of trifluoromethyl ketone inhibitor is chemically more stable than that (–O–CO–R) of acyl-enzyme inhibitor.

In 1992, Edwards and co-workers at Zeneca disclosed the crystal structure of the complex of substrate-based inhibitor having α-ketoheterocyclic moiety and porcine pancreatic elastase, demonstrating the importance of the structure of the binding sites P1, P2, and P3 side chains in the inhibitors (Fig. 2).[4] This led us to introduce substituent (OR) on benzoxazole ring, by which a better fitting to S1′ subsite of HNE could be achieved. The compounds that lack of the amino acid moiety (Val or Val-Pro in Fig. 2), may have some potent activities with the presence of fitting substituent (OR) and also expected to enhance the metabolic stability.

Table 1. Substituent effect on HNE inhibitory activity



Compd[a]	–OR	IC_{50} (μM)[b]	Compd[a]	–OR	IC_{50} (μM)[b]
4a	–H	>10	**4h**		7.8
4b	–OH	>10	**4i**		>10
4c		0.45	**4j**		>10
4d		>10	**4k**		>10
4e		>10	**4l**		>10
4f		>10	**4m**		>10
4g		>10	**4n**		>10

[a]All compounds are greater than 90% of diastereomer with the *S* configuration at the stereogenic center at α to the ketone carbony group.
[b]Inhibition of HNE-catalyzed hydrolysis of the synthetic substrate Suc-Ala-Pro-Ala-MCA.[6]

Chemistry

The simplest unsubsituted benzoxazole derivative (**4a,** Table 1) was prepared by the reported method.[4] The synthetic routes for the *O*-substituted benzoxazole derivatives (**4b–n**) are summarized in Scheme 1. Thus, 4-hydroxybenzoxazole derivative (**2**) derived from **1** was prepared by Pinner condensation[5] between 2-amino-resorcinol with cyanohydrin. Phenolic hydroxy group was selectively protected with *tert*-butyldimethylsilyl group (TBS), and then oxidation of secondary hydroxy group with Dess–Martin periodinane (DMP) gave **3**. After removing of TBS group, esterification or etherification were performed by the usual manner to afford **4b–n**. Scheme 2 shows the preparation of carboxylic acid derivatives (**9a–n**). Deprotection of the Z-group in **3** provided **5**, which was coupled with Z-Pro–OH or Z-Val-Pro–OH to afford **6a** or **6b**. After removing the Z-group, the terminal-ester group was introduced by the condensation with half-esters to give **7a** and **7b**. After oxidation of secondary alcohol of **7a** and **7b** with DMP, transformation of TBSO group to ester or ether group was then performed in a same manner as the methods described in the Scheme 1. Finally, treatment with TFA gave the desired compounds (**9a–n**).

Results and Discussion

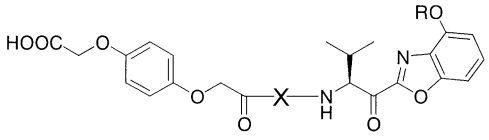
Substituent of benzoxazole ring

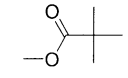
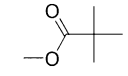
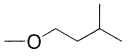
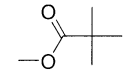
Effects of the substituents on the benzoxazole ring were evaluated (Table 1). Various ester- and ether-based substituents were incorporated into the 4-position of the benzoxazole ring. Among them, pivaloyl ester (**4c**) and isopentyl ether (**4h**) groups were found to increase the activity in comparison with unsubstituted compound **4a**. Unsaturation (**4i**), migration of methyl group (**4j**), and exchange of secondary carbon atom with nitrogen atom (**4k**) of the isopropyl group in compound **4h** lost the activity at dose of 10 μM. Aryl alkyl ethers (**4d–f**) are also not effective substituents. Furthermore, the introduction of hydrophilic moieties (**4b, 4m**, and **4n**) resulted in loss the activity. These results suggest that at the active site of the enzyme, there may be present a lipophilic pocket that accommodates the substituents at the 4-position of the benzoxazole ring.

Effect of amino acid residue

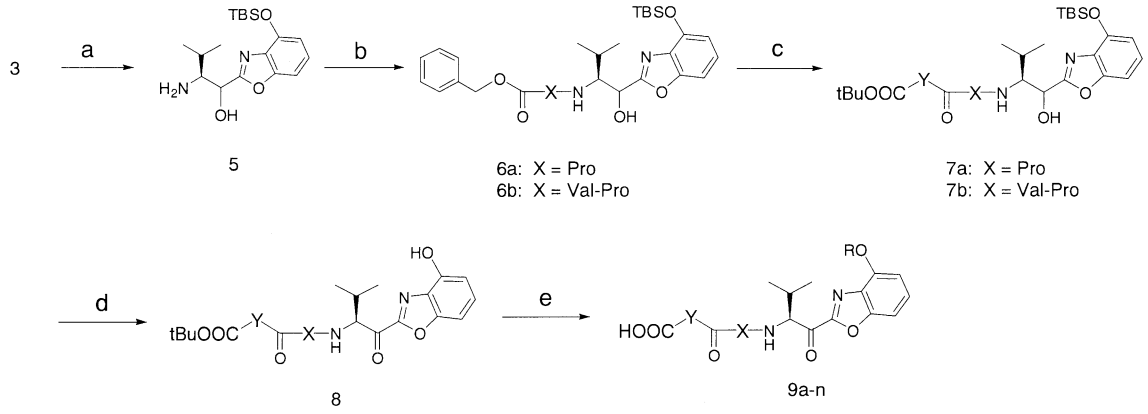
Several advanced derivatives were prepared in which amino acid residues have incorporated (Table 2). Phenoxyacetic acid moiety was also introduced to increase water-solubility, which did not affect the parent activity (**4c** vs **9a**). Although an incorporation of Pro-residue (**9b** and **9c**) did not increase in the in vitro activity, the in vivo activities of these series are improved in some extent at a high dose (100 mg/kg). The presence of Val residue dramatically increased in the in vitro activity (**9d**). The importance of the Val residue in this position for the in vivo activity was in accordance with the previously reported results for peptide trifluoromethyl ketone inhibitors.[8] However, this modification did not increase the in vivo potency; this indicates that the decrease of the chain length in the peptide possibly increase the metabolic stability. However, undesirable toxicity (*muscular stiffness in hamsters*) was observed when **9b** and **9c** were administrated intravenously at a

Table 2. Effect of amino acid residue on HNE inhibitory activity



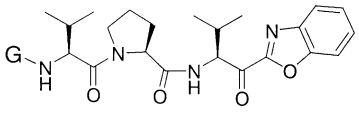
Compd[a]	–OR	–X–	IC_{50} (μM)[b]	% inhibition after iv bolus administration[c]	
				100 mg/kg	30 mg/kg
9a		—	0.13	74	13
9b		–Pro–	0.23	96	66
9c		–Pro–	0.29	99	−16
9d		–Val-Pro–	0.0089	99	73
9e	—H	–Val-Pro–	0.0076	98	56

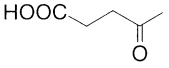
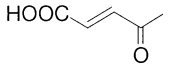
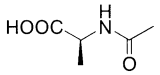
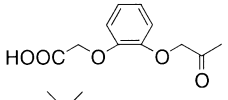
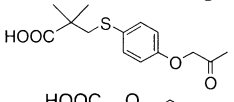
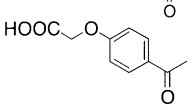
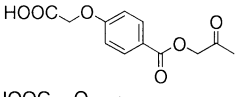
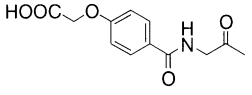
[a]All compounds are greater than 90% of diastereomer with the *S* configuration at the stereogenic center at α to the ketone carbony group.
[b]Inhibition of HNE-catalyzed hydrolysis of the synthetic substrate Suc-Ala-Pro-Ala-MCA.[6]
[c]Inhibition of HNE-induced lung hemorrhage in hamster.[7]



Scheme 2. (a) H_2, $Pd(OH)_2$, AcOEt; (b) Z-Pro-OH or Z-Val-Pro-OH, WSC, Py; (c) (i) H_2, $Pd(OH)_2$, AcOEt; (ii) *t*BuOOC-Y-COOH, WSC, Py; (d) (i) DMP, CH_2Cl_2; (ii) TBAF, THF; (e) (i) R-Br or R-I, NaH, DMF or RCOCl, Et_3N, CH_2Cl_2; (ii) TFA, CH_2Cl_2.

Table 3. Effect of terminal acid residue on HNE inhibitory activity



Compd[a]	–G	IC_{50} (μM)[b]	% inhibition after iv bolus administration[c]		
			100 mg/kg	30mg/kg	10 mg/kg
9f		0.066		61	
9g		0.072			ND[d]
9h		0.28		39	
9i		0.0072	90	48	37
9j		0.0035	99	86	30
9k		0.0074		60	15
9l		0.013		75	12
9m		0.033		99	46

[a]All compounds are greater than 90% of diastereomer with the *S* configuration at the stereogenic center at α to the ketone carbony group.
[b]Inhibition of HNE-catalyzed hydrolysis of the synthetic substrate Suc-Ala-Pro-Ala-MCA.[6]
[c]Inhibition of HNE-induced lung hemorrhage in hamster.[7]
[d]Due to the low solubility, inhibitory activity was not determined.

dose of 100 mg/kg, but such behavior was not observed for **9d**. This toxicity seems to be caused from the lack of Val residue. This observation directed us to fix on the X as Val-Pro. As the introduction of the pivaloyl substituent was not effective for the Val-Pro type (**9d** vs **9e**), we then focused on the modification of the terminal acidic moiety (Table 3).

Effect of terminal acid residue

Table 3 shows that the introduction of an aromatic ring in the terminal acidic moiety is preferred for the in vitro inhibitory activity. The substituent-position of carboxylic acid moiety of benzene ring was not crucial for the activities (**9e** in Table 2 vs **9i** in Table 3). Replacing

the oxygen atom of **9e** with sulfur and introduction of methyl group at acetic acid group for increasing the bulkiness as in **9j** resulted in similar potencies in both in vitro and in vivo activities. The in vitro inhibitory activities of the ester (**9l**) and the amide (**9m**) derivatives were less potent than that of **9e**. However, the in vivo activity of **9m** was the best in this series; HNE-induced lung hemorrhage in hamsters was almost completely inhibited at a dose of 30 mg/kg. The presence of amide bond would increase a chemical stability or have better pharmacokinetic properties than other synthesized compounds. For further evaluation, both pharmacological and synthetic studies on this amide compound **9m** and its derivatives are in progress.

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7. Test compound was administrated into jugular vein 5 min before an intratracheal instillation of HNE (25 units). One hour after the induction of lung injury, BALF was collected and hemorrhage in BALF was estimated by measuring of absorbance at 414 nm.

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