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Synthesis and Structure–Activity Relationships of a New Class of 1-Oxacephem-Based Human Chymase Inhibitors

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Abstract—1-Oxacephem derivatives were synthesized and evaluated as a novel series of chymase inhibitors. Structure–activity relationship studies of 1-oxacephems led to compound **34**, which exhibited 6 nM inhibition of human chymase and high selectivity for human chymase compared to other serine enzymes.

Introduction

Human chymase is a chymotrypsin-like serine protease that is stored in the secretory granules of mast cells.[1] Although the physiological and pathological roles of chymase have not been fully elucidated, this enzyme has been shown to convert angiotensin I to angiotensin II with greater efficiency than angiotensin I converting enzyme.[2] Chymase has also been shown to participate in histamine release from mast cells,[3] activation of precursor interleukin-1β,[4] and cleavage of type I procollagen[5] and progelatinase B.[6] Thus, chymase is speculated to play an important role in cardiovascular diseases and chronic inflammation following fibrosis such as cardiac, renal, and pulmonary fibrosis.[7] Chymase inhibitors[8] are thought to be potentially useful as tools for elucidating the physiological function of chymase and therapeutic agents.

Screening of the Shionogi compound collection led to identification of the 1-oxacephem derivative **1**[9] as a chymase inhibitor (IC_{50} 0.25 μM, Fig. 1), which was prepared for a project on Latamoxef,[10] an antibacterial agent developed at our company. This is the first report of β-lactam compounds inhibiting human chymase. We describe herein the structure–activity relationships by chemical modifications at the 3′-, 4- and 7β-positions of the 1-oxacephem nucleus.

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Chemistry

1-Oxacephem derivatives were prepared as shown in Schemes 1 and 2. Amine **2** was prepared by a procedure established in our laboratories.[11] First, 7β-substituted 1-oxacephems **3**–**13** were obtained by treatment with amine **2** and a variety of acid chlorides prepared from the corresponding acids by a usual method (oxalyl chloride, DMF), in the presence of pyridine. Next, 4-substituents **15**–**25** were prepared in the following way. Deprotection ($AlCl_3$, anisole, CH_2Cl_2 99%) of diphenylmethylester **1** provided compound **14**. Esterification of Na salt of **14** with a variety of alkyl halides and amidation of mixed anhydride prepared from **14** with three amines gave 4-substituted 1-oxacephem esters **15**, **16**, **20**–**25** and amides **17**–**19**, respectively. Next, 3′-substituents **27**–**32** were obtained by the following procedures. Reduction of **21** (Mg, CH_2Cl_2–AcOH) gave exomethylene **26** (47%) and 3-methyl **27** (32%). Protection of phenol **26** (CCl_3COCl, pyridine, CH_2Cl_2), dichlorination of exomethylene (Cl_2, CCl_4, hν) followed by dehydrochlorination and deprotection ($NaHCO_3$, H_2O, 100% from **26**) provided chloromethyl **28**.[12] 3′-Substituted 1-oxacephems **29**–**32** were obtained by treatment with **28** and the corresponding thiols or tetrazol in the presence of *i*-Pr_2NEt. The most potent compound **34** was prepared from **11** by the same procedures described above (Scheme 2).

Results and Discussion

First, we examined the substituent effects on the phenyl group at 7β-position as shown in Table 1.[13] In the *para*-

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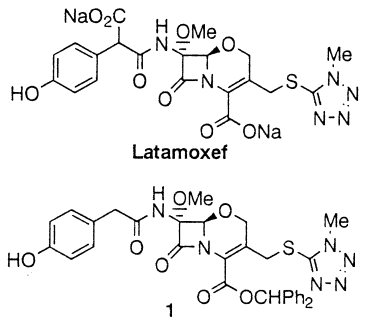
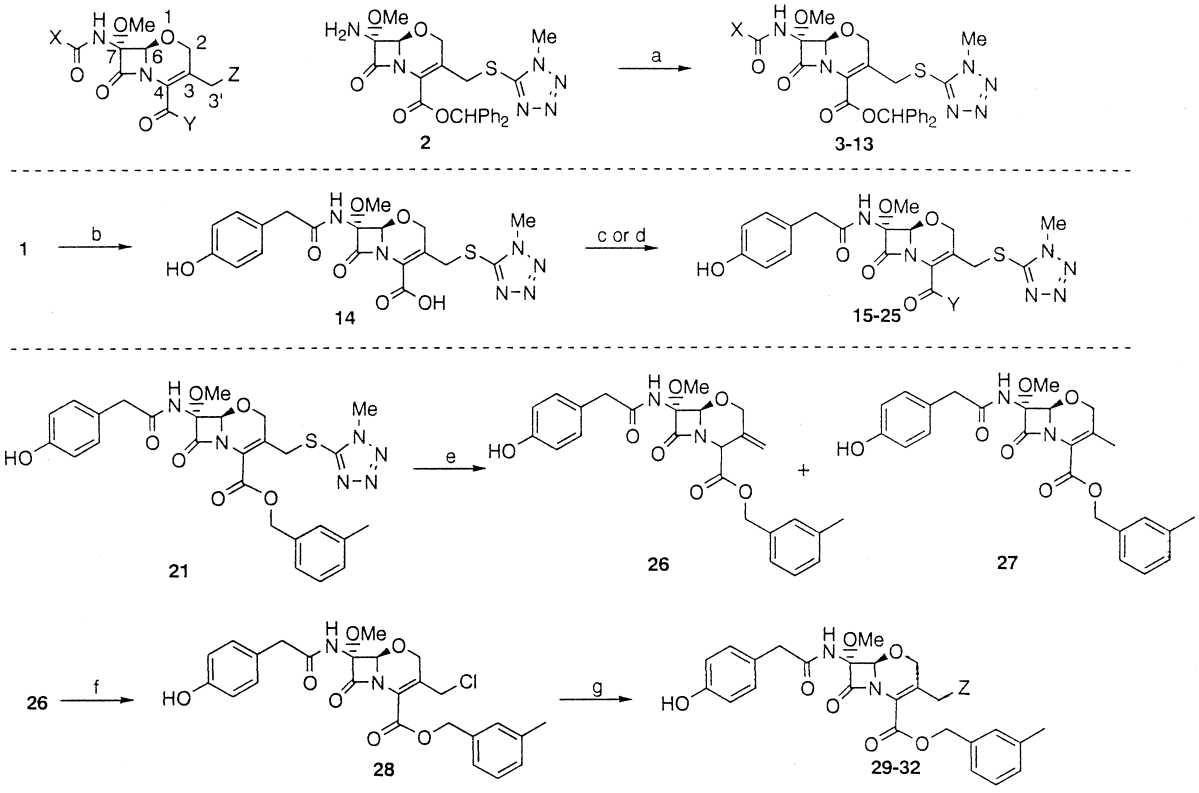


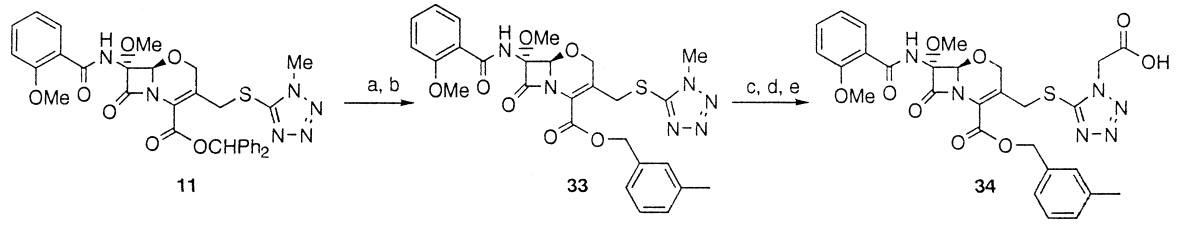
Figure 1.

substituted phenylacetamide derivatives, unsubstituted compound **3** and hydrophilic substituents **4** and **5** showed approximately the same activity as the lead compound **1**. Hydrophobic substituents such as bromine **6** and phenyl **7** remarkably decreased potency. The hydroxy group **1** appears optimal (IC_{50} 0.25 μM). Among the methoxy-substituted benzamide derivatives, *ortho*-substituent **11** was more potent than the corresponding *para*- **9** and *meta*-substituents **10**. Substituents at the *ortho*-position, such as chlorine **12**, methoxycarbonyl **13** and hydrogen (unsubstituted) **8** decreased the activity moderately. *ortho*-Methoxy **11** gave the highest IC_{50} value (0.55 μM) among the benzamide derivatives.

Next, a substituent at the 4-position was optimized for the 7β-*para*-hydroxyphenylacetamide as shown in Table 2. Ester derivatives **1**, **15** and **16** were more potent than amide derivatives **17**–**19**. Especially, esters **1** and **16** displayed 44- and 110-fold increase compared to the corresponding amides **17** and **18**, respectively. Because 4-benzyl ester **16** was the most active, we prepared a variety of substituted benzyl esters. Introduction of a methyl group into *meta*-position **21** in 4-benzyl ester derivative resulted in a threefold increase of potency over that of **16**, while introduction into the *para*- and *ortho*-position **20** and **22** led to a mild decrease. With regard to *meta*-

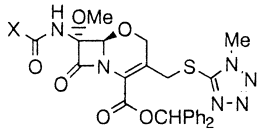


Scheme 1. Reagents and conditions: (a) XCOCl, pyridine, CH_2Cl_2; (b) $AlCl_3$, anisole, CH_2Cl_2, 99%; (c) (i) sodium 2-ethylhexanoate, MeOH–EtOAc; (ii) RCH_2Br or MeI, DMF; (d) (i) *t*-BuCOCl, Et_3N, THF; (ii) NMM, HNRR′; (e) Mg, CH_2Cl_2–AcOH, **26** (47%), **27** (32%); (f) (i) CCl_3COCl, pyridine, CH_2Cl_2; (ii) Cl_2, CCl_4, hv; (iii) $NaHCO_3$, H_2O, 100%; (g) ZH, *i*-Pr_2NEt, MeCN.



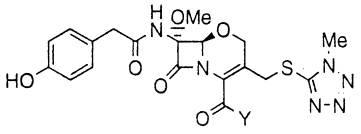
Scheme 2. Reagents and conditions: (a) $AlCl_3$, anisole, CH_2Cl_2; (b) (i) sodium 2-ethylhexanoate, MeOH–EtOAc; (ii) $BrCH_2C_6H_4$-3-Me, DMF; (c) Mg, CH_2Cl_2–AcOH; (d) (i) Cl_2, CCl_4, hv; (ii) $NaHCO_3$, H_2O; (e) 5-mercapto-1-tetrazoleacetic acid, Et_3N, MeCN.

Table 1. Modifications at 7β-position



Compound	X	IC_{50} (μM)	Compound	X	IC_{50} (μM)
1	$CH_2C_6H_4$-4-OH	0.25	**8**	C_6H_5	1.40
3	$CH_2C_6H_5$	0.30	**9**	C_6H_4-4-OMe	>10
4	$CH_2C_6H_4$-4-OMe	0.30	**10**	C_6H_4-3-OMe	7.31
5	$CH_2C_6H_4$-4-NMe_2	0.41	**11**	C_6H_4-2-OMe	0.55
6	$CH_2C_6H_4$-4-Br	6.80	**12**	C_6H_4-2-Cl	1.70
7	$CH_2C_6H_4$-4-Ph	>10	**13**	C_6H_4-2-CO_2Me	3.50

Table 2. Modifications at 4-position



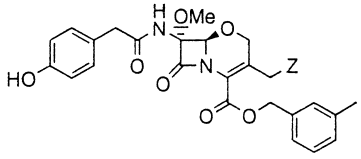
Compound	Y	IC_{50} (μM)	Compound	Y	IC_{50} (μM)
1	$OCHPh_2$	0.25	**20**	$OCH_2C_6H_4$-4-Me	0.17
15	OMe	1.60	**21**	$OCH_2C_6H_4$-3-Me	0.05
16	OCH_2Ph	0.14	**22**	$OCH_2C_6H_4$-2-Me	0.43
17	$NHCHPh_2$	11.0	**23**	$OCH_2C_6H_4$-3-Br	0.07
18	$NHCH_2Ph$	15.6	**24**	$OCH_2C_6H_4$-3-CF_3	0.05
19	$N(CH_2)_4$	>10	**25**	$OCH_2C_6H_4$-3-CO_2Me	1.65

substituents, methyl substituent **21** gave approximately the same activity as bromine **23** and trifluoromethyl **24**. Methoxycarbonyl group **25** led to a 33-fold loss of potency versus **21**. *meta*-Methyl **21** showed the best result (IC_{50} 0.05 μM) among the 4-substituted derivatives. 4-Carboxylate as shown in latamoxef (Fig. 1) is necessary for expression of the antibacterial activity, but also dramatically decreases the potency against human chymase. On the other hand, 4-benzyl ester increased the activity against human chymase and led to loss of antibacterial activity (data not shown).

Finally, in order to enhance the potency of **21**, we modified the 3′-substituents (Table 3). Compound **21** was more active than compounds **27**, **28**, **31** and **32**, but roughly showed the same potency as compounds **29** and **30**. 3′-Thiotetrazoleacetic acid **29** was designed and prepared in order to improve water solubility and consequently facilitate in vivo evaluation.

In order to investigate the inhibitory mechanism of 1-oxacephems against human chymase, we performed a series of kinetic studies.[14] Judging from the results of kinetic studies and reports concerning β-lactam inhibitors of human leukocyte elastase,[15,16] we assumed that the inhibition mechanism of 1-oxacephems against human chymase was as presented in Figure 2 which shows that the active site serine residue (serine 195) in the enzyme approaches the β-lactam ring of 1-oxacephem, followed by generation of an acyl-enzyme.

Table 3. Modifications at 3′-position



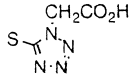
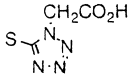
Compound	Z	IC_{50} (μM)	Compound	Z	IC_{50} (μM)
21		0.05	**30**		0.08
27	H	>10	**31**		0.44
28	Cl	0.15	**32**		4.00
29		0.07			

Table 2 shows that amide derivatives **17** and **18** were less potent than 4-ester derivatives **1** and **16**. Two reasons can be considered for this: (1) a more electron-withdrawing character of the ester group than the amide group may facilitate the nucleophilic attack of active serine 195 to β-lactam carbonyl and (2) 4-electron-rich amide carbonyl or amide-NH may prevent hydrogen

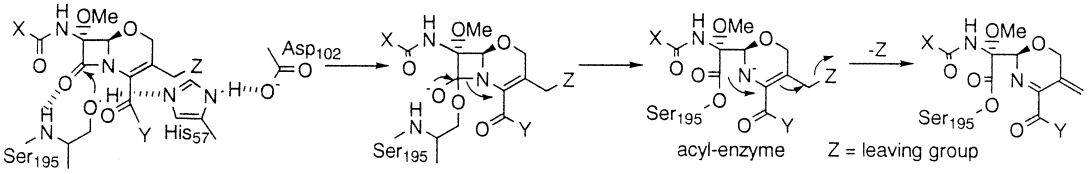


Figure 2. Proposed mechanism for inhibition of human chymase by 1-oxacephem.

Table 4. Selectivity of **34** as an inhibitor of human chymase compared to other serine proteases

Enzyme	IC_{50} (μM)	Enzyme	IC_{50} (μM)
Chymase	0.006	Trypsin	0.44
α-Chymotrypsin	0.16	Elastase	>10
Cathepsin G	0.23	Plasmin	>10
Thrombin	0.43		

abstraction of serine 195 by histidine 57 or the approach of serine 195 to β-lactam carbonyl.[17] Figure 2 depicts the mechanism at work when the 3′-substituent Z is a good leaving group. According to Table 3, when 3′-substituent Z was not the leaving group (hydrogen, **27**), no activity was observed. This result supports the proposed mechanism.

Considering the match–mismatch between 3′, 4 and 7β-substituents, several hybrid compounds were prepared based on the results presented in Tables 1–3. Fortunately, we found hybrid compound **34** to possess the highest potency (IC_{50} 6 nM) and to be 40-fold more active than lead compound **1** (Scheme 2). In compound **34**, the combination of substituents at 3′, 4 and 7 positions may match synergetically. Enzymatic work showed that **34** was an extremely selective inhibitor, causing weak or no inhibition of several other serine proteases (Table 4).[18] In addition, the Na salt of **34** possessed water solubility (500 mg/mL). Consequently, it was considered to be suitable for in vivo evaluation.

Conclusion

We have described here the synthesis of 1-oxacephem derivatives and their inhibitory activities against human chymase. We found that compound **34** possesses high potency and selectivity against human chymase.

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References and Notes

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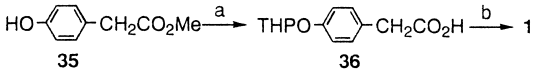
9. Lead compound **1** was prepared as follows: (a) (i) dihydropyrane, TsOH, CH_2Cl_2; (ii) NaOH, acetone, 94% from **35**; (b) (i) $POCl_3$, CH_2Cl_2; (ii) **2**, pyridine; (iii) HCl–acetone, 76% from **36**.



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13. The human chymase assay was performed as follows: first, human chymase was purified according to the method of Takai (ref, Takai, S.; Siota, N.; Sakaguchi, M.; Muraguchi, H.; Matsumura, E.; Miyazaki, M. *Clin. Chim. Acta* **1997**, *265*, 13). The purified chymase was preincubated with test compounds dissolved in DMSO at 37 °C for 30 min in 0.1 M Tris–HCl (pH 8.0) containing 1.8 M NaCl, after then the chymase reaction was started by adding succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilides (Sigma Chemical Co.). The change of absorbance was measured at 405 nm after 2 h incubation at 37 °C. The IC_{50} value was calculated from the inhibition of *p*-nitroaniline formation at each concentration of the test compound.
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16. See ref 8d with respect to the catalytic triad (Ser-195, His-57, Asp-102) in human chymase.
17. Cephalosporin ester and amide sulfones were tested to determine the structure–activity relationships for inhibition of

human leukocyte elastase (ref, Finke, P. E.; Ashe, B. M.; Knight, W. B.; Maycock, A. L.; Navia, M. A.; Shah, S. K.; Thompson, K. R.; Underwood, D. J.; Weston, H.; Zimmerman, M.; Doherty, J. B. *J. Med. Chem.* **1990**, *33*, 2522).

18. The inhibitory effects of compound **34** on the enzymatic activities of seven serine proteases were evaluated using the purified enzymes and chromogenic substrates. The enzymes and substrates used here were as follows: *N*-succinyl-Ala-Ala-Pro-Phe-pNA (Bachem) for bovine pancreatic α-chymotrypsin (Sigma) and human cathepsin G (Wako); chromozyme TH (Boehringer Mannheim) for human thrombin (Sigma); *N*-succinyl-Ala-Ala-Phe-Arg-pNA (Bachem) for bovine pancreatic trypsin (Sigma); *N*-succinyl-Ala-Ala-Val-pNA (Bachem) for human neutrophil elastase (Athens Research and Technology, Inc.); chromozym PL (Boehringer Mannheim) for human plasmin (Sigma). The assay buffer used here was as follows: 50 mM Tris–HCl (pH = 8.0) containing 2 mM $CaCl_2$ for α-chymotrypsin, trypsin and elastase; 50 mM Tris–HCl (pH = 7.5) containing 2 mM $CaCl_2$ for cathepsin G; 50 mM Tris–HCl (pH = 7.5) containing 50 mM NaCl for plasmin; 0.1 M Tris–HCl (pH = 8.0) containing 10 mM $CaCl_2$ and 0.1 M NaCl for thrombin.