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1-Oxacephem-Based Human Chymase Inhibitors: Discovery of Stable Inhibitors in Human Plasma

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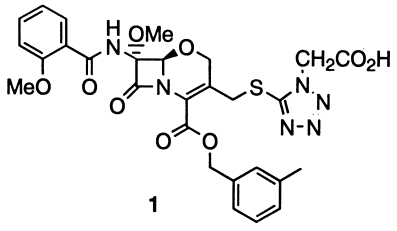
Abstract—1-Oxacephem derivatives were evaluated as a novel series of chymase inhibitors. The structure–activity relationship studies of 1-oxacephems led to compounds **15**, which exhibited 27 nM inhibition of human chymase and improvement of stability in human plasma ($t_{1/2}$ 1.5 h).

Introduction

In the preceding paper,[1] we reported a novel series of 1-oxacephem-based human chymase inhibitors as potential therapeutic agents for cardiovascular diseases and chronic inflammation following fibrosis such as cardiac, renal, and pulmonary fibrosis.[2] 1-Oxacephem derivative **1** exhibited potent and selective inhibition against human chymase. However, in vivo evaluation of candidate **1** (IC_{50} 6 nM) was limited by its lability in human plasma ($t_{1/2} < 10$ min). The results of in vivo assay of non-peptidyl chymase inhibitors[3] have been scarcely reported, and one reason is their lability in human plasma. In order to find a drug candidate, it is essential to enhance the stability of the active compound in human plasma. We thus studied to improve stability in human plasma while maintaining high activity against human chymase.



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Chemistry

1-Oxacephem derivatives **1** and **7**–**13** were prepared by the same synthetic route as described in the preceding paper.[1] 1-Oxacephems **14**–**19** bearing 4-carboxyphenylmethyl ester at the 4-position were prepared starting from **3** (Scheme 1). Amine **3**[1] was treated with a variety of benzoyl chlorides **2a**–**f** to give 7β-amides **4a**–**f**. 4-Diphenylmethyl esters **4a**–**f** were deprotected by $AlCl_3$ and anisole to yield acids **5a**–**f**. Compounds **6a**–**f** were obtained by esterification of Na salt of **5a**–**f** with diphenylmethyl 4-bromomethylbenzoate, which was prepared by treatment with 4-bromomethylbenzoic acid and diphenyldiazomethane. Deprotection of diphenylmethyl esters **6a**–**f** (TFA, anisole, CH_2Cl_2) provided 1-oxacephem derivatives **14**–**19**.

Results and Discussion

Two paths may be suggested for the destruction of 1-oxacephems in human plasma. One is a simple hydrolysis of the 4-ester, and the other is cleavage of the β-lactam ring shown in Figure 1. We assumed the latter was the major destruction path, because our preliminary investigation showed that no carboxylic acid was detected when oxacephem ester was treated in human plasma. Figure 1 shows that a nucleophile such as the hydroxy group in a serine residue in various proteins in human plasma attacks the β-lactam carbonyl of 1-oxacephem, leading to cleavage of the β-lactam ring and suggests inhibition against human chymase and destruction in

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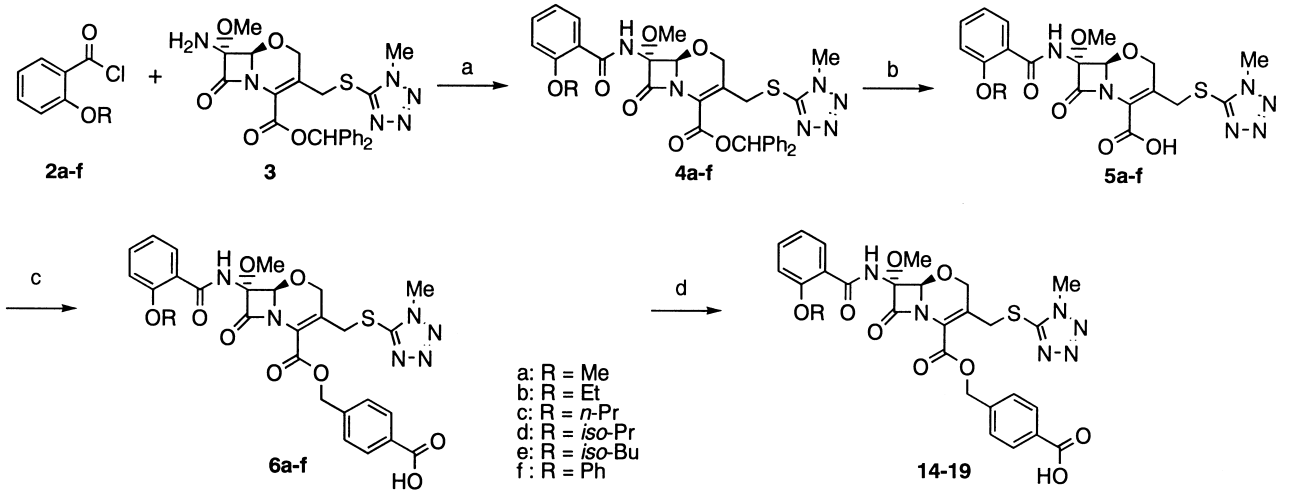


Scheme 1. Reagents and conditions: (a) pyridine, CH_2Cl_2; (b) $AlCl_3$, anisole, CH_2Cl_2; (c) (i) $NaHCO_3$, H_2O–MeOH; (ii) $BrCH_2C_6H_4$-4-CO_2CHPh_2, DMF; (d) TFA, anisole, CH_2Cl_2.

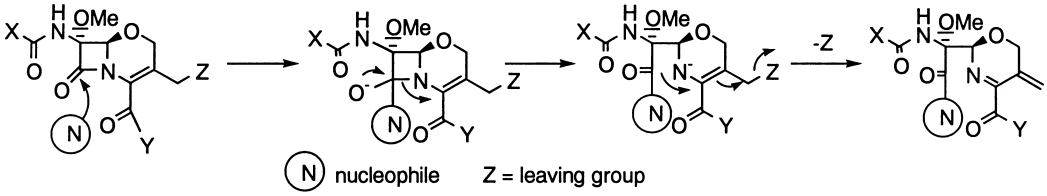
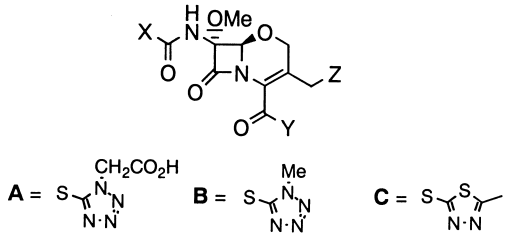


Figure 1. Proposed mechanism for destruction of 1-oxacephems in human plasma.

Table 1. Effects of modifications at the 3′-, 4- and 7β-positions



Compound	X	Y	Z	Stability (%)[a]	IC_{50} (μM)
1	C_6H_4-2-OMe	$OCH_2C_6H_4$-3-Me	**A**	11	0.006
7	$CH_2C_6H_4$-4-OH	$OCH_2C_6H_4$-3-Me	**A**	5	0.072
8	$CH_2C_6H_4$-4-OH	$OCH_2C_6H_4$-3-Me	**B**	10	0.050
9	$CH_2C_6H_4$-4-OH	$OCH_2C_6H_4$-3-Me	**C**	13	0.077
10	$CH_2C_6H_4$-4-OH	$OCH_2C_6H_4$-3-Me	H	59	> 10
11	$CH_2C_6H_4$-4-OH	$OCHPh_2$	**B**	40	0.25
12	$CH_2C_6H_4$-4-NMe_2	$OCHPh_2$	**B**	53	0.41
13	C_6H_4-2-OMe	$OCHPh_2$	**B**	73	0.55
14	$C_6H_4$2-OMe	$OCH_2C_6H_4$-4-CO_2H	**B**	26	0.019

[a]The percent of test compounds remaining in human plasma after 30 min was observed.

human plasma via the same mechanism as cleavage of β-lactam ring. Accordingly, increase of potency is considered to be inversely proportional to improvement of stability in human plasma. In considering this problem, we investigated the enhancement of stability without drastic decrease of the potency by modifications at the 3′-, 4- and 7β-positions (Tables 1 and 2).[4,5]

First, we investigated the substituent effect at the 3′-position of oxacephem as shown in Table 1. When substituent Z was not the leaving group, the stability improved (**10** versus **7**, **8** and **9**). This result is in accordance with our proposed destruction mechanism of 1-oxacephems in human plasma (Fig. 1). However, compound **10** showed no significant inhibition against

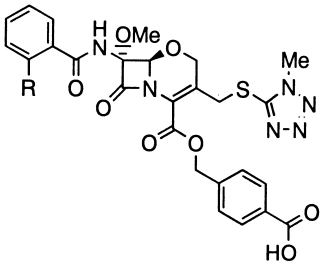
human chymase. Consequently, modifications at the 3′-position did not lead to enhancement of stability with maintenance of the potency, and we guessed that modifications at 3′-position were not suitable for achieving our purpose.

Next, 4-substituents were evaluated. 4-Ester **1** was much less stable in human plasma than the corresponding 4-sodium carboxylate. The electronegative effect of the 4-ester group has been known to increase reactivity at the β-lactam carbonyl group and we think this is the reason for the poor stability of compound **1**. The 4-ester group is needed to increase the potency as shown in the preceding report.[1] We next investigated a variety of 4-esters and found that the diphenylmethyl ester group enhanced stability (**8** versus **11**, **12** and **13**). However, these compounds **11**, **12** and **13** displayed 40–90-fold less potency than benzyl ester **1** presumably owing to steric hindrance. Further investigation showed 4-*para*-carboxyphenylmethyl ester **14** to be effective for improvement of stability, although this ester was not as good as diphenylmethyl ester as a stabilizing group. Compound **14** was only threefold less potent than compound **1**.

Finally, a substituent at the 7β-position was optimized for 4-*para*-carboxyphenylmethyl ester oxacephems (Table 2). In order to enhance the stability of **14**, we modified *ortho*-alkoxy substituents. Ethoxy derivative **15** possessed good potency and stability in human plasma ($t_{1/2}$ 1.5 h). Because there are only minor differences between compounds **14**–**19** as regards the inhibitory activity against human chymase, we supposed that *ortho*-substituents especially play an important role in stability in human plasma.

Additional enzymatic work showed that compound **15** was a selective inhibitor, displaying weak or no inhibition of several other serine proteases (Table 3).[4]

Table 2. Modification at 7β-position



Compound	R	Stability (%)[a]	IC_{50} (μM)
14	OMe	26	0.019
15	OEt	87 (1.5 h)[b]	0.027
16	OPr-*n*	26	0.024
17	OPr-*iso*	42	0.037
18	OBu-*iso*	39	0.026
19	OPh	63	0.057

[a]The percent of test compounds remaining in human plasma after 30 min was observed.
[b]The number in parentheses represents the half-life period.

A model for the inhibition of compound **15** of human chymase, using atomic coordinates from the published X-ray structure of the enzyme,[6] is shown in Figure 2.[7,8] In this model, the inhibitor **15** is bound to the active site of the enzyme with hydroxy of Ser195 and the resulting hemiketal oxygen is in the oxyanion hole formed by the backbone amide NH of Ser195 and Gly193. The benzamide substituent at the 7β-position is fully enclosed by the residues of the S1 pocket and capped with the side chain of Phe191. The benzylester substituent at the 4-position occupies the hydrophobic prime site region

Table 3. Selectivity of **15** as an inhibitor of human chymase compared to other serine proteases

Enzyme	IC_{50} (μM)	Enzyme	IC_{50} (μM)
Chymase	0.027	Trypsin	2.1
α-Chymotrypsin	0.16	Elastase	9.5
Cathepsin G	0.28	Plasmin	>10
Thrombin	3.6		

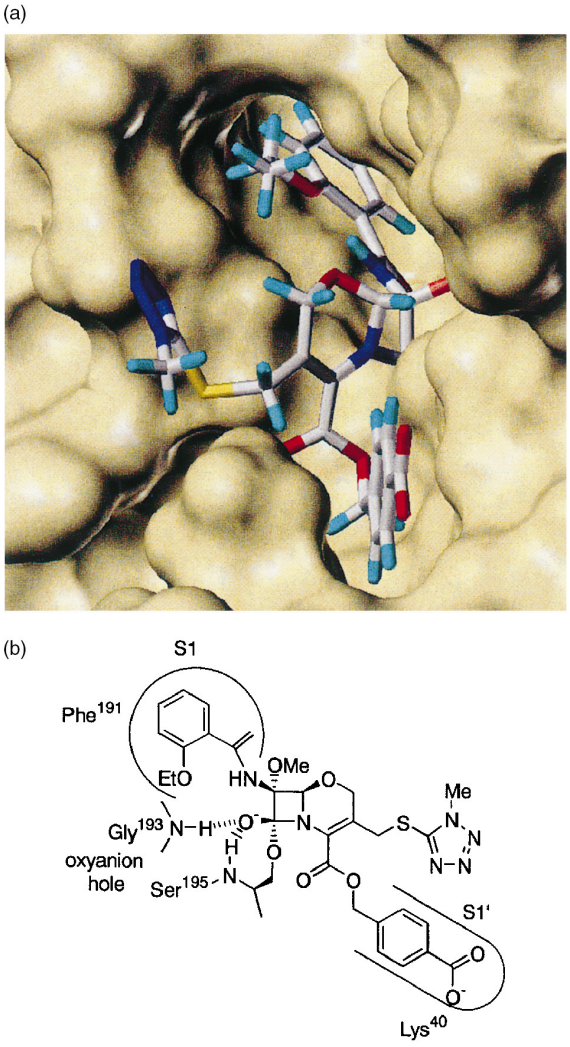


Figure 2. Docking model of inhibitor **15** into human chymase. (a) Surface representation. (b) Schematic drawing.

(S1′) of the enzyme. The terminal carboxylic acid electrostatically interacts with the side chain of Lys40. This model accounts for the potency of compound **15** against human chymase.

Conclusion

We described here the discovery of 1-oxacephem-based human chymase inhibitors with both high inhibitory activities and moderate stability in human plasma. As a compromise between potency and stability, 4-*para*-carboxyphenylmethyl ester was found to be optimum. Compound **15** was found to possess high potency against human chymase (IC_{50} 27 nM) and stability in human plasma ($t_{1/2}$ 1.5 h), and accordingly, is a promising candidate for in vivo assay.

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References and Notes

1. Aoyama, Y.; Uenaka, M.; Konoike, T.; Iso, Y.; Nishitani, Y.; Kanda, A.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2397.
2. (a) Shiota, M.; Fukamizu, A.; Takai, S.; Okunishi, H.; Murakami, K.; Miyazaki, M. *J. Hypertens*. **1997**, *15*, 431. (b) Hamada, H.; Terai, M.; Kimura, H.; Hirano, K.; Oana, S.; Niimi, H. *Am. J. Respir. Crit. Care. Med*. **1999**, *160*. 1303. (c) Takai, S.; Yuda, A.; Jin, D.; Nishimoto, M.; Sakaguchi, M.; Sasaki, S.; Miyazaki, M. *FEBS Lett*. **2000**, *467*, 141.
3. (a) Hayashi, Y.; Iijima, K.; Katada, J.; Kiso, Y. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 199. (b) Groutas, W. C.; Schechter, N. M.; He, S.; Yu, H.; Huang, P.; Tu, J. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 2199. (c) Iijima, K.; Katada, J.; Hayashi, Y. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 413. (d) Fukami, H.; Okunishi, H.; Miyazaki, M. *Curr. Pharm. Des*. **1998**, *4*. 439 and references therein.
4. See ref 1 for the enzyme assay.
5. The stability assay in human plasma was performed as follows. Test compounds dissolved in DMSO were added at 20 μg/mL to human plasma and incubated at 37 °C. Aliquots were removed at intervals of 30 min, quenched with threefold volume of AcOH:MeOH (1:1) or AcOH:MeCN (1:1), and analyzed by reverse-phase HPLC after removing proteins. The disappearance of compound **15** was then monitored for the half-life period and the results fitted a first-order decay curve from which the reported $t_{1/2}$ value was derived.
6. Pereira, P. J. B.; Wang, Z. M.; Rubin, H.; Huber, R.; Bode, W.; Schechter, N. M.; Strobl, S. *J. Mol. Biol*. **1999**, *286*, 163. (PDB code, 1pjp)
7. The docking study for the binding of cephalosporin ester to human leukocyte elastase was performed at Merck (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).
8. Molecular modeling was performed using the SYBYL6.6 software package. The three-dimensional model of compound **15** was constructed based on the published X-ray structure of an oxacephem compound (ref, Shiro, M.; Nakai, H.; Onoue, H.; Narisada, M. *Acta Cryst*. **1980**, *B36*, 3137). The model of inhibitor **15** was manually docked into the active site of human chymase on the basis of the structure–activity relationships of oxacephem derivatives against human chymase and the docking study for the binding of cephalosporin ester to human leukocyte elastase.[7] The resulting hemiketal intermediate (human chymase–**15** complex) was energy-minimized using the Tripos force field.