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Structure–Activity Relationship Studies of Chloromethyl Ketone Derivatives for Selective Human Chymase Inhibitors

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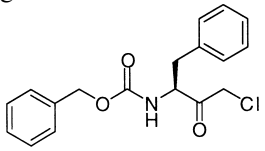
Abstract—Based on the SAR study of a classical chloromethyl ketone derivative, Z-PheCH_2Cl **1**, a series of compounds were synthesized. Among all the derivatives, compound **21** was found to be a potent human chymase inhibitor with no inhibitory activity against human leukocyte cathepsin G.

Chymase is a chymotrypsin-like serine protease found in mast cells.[1] Recent studies have demonstrated that chymase generates angiotensin II (Ang II) from its inactive precursor angiotensin I in several species including humans, dogs and hamsters.[2,3] The local, but not the systemic, generation of Ang II is suggested to be involved in the vascular remodeling and hence, implicated in the pathogenesis of several diseases such as atherosclerosis, restenosis after angioplasty or heart failure.[4–6] Chymase is shown to be one of the primary enzymes responsible for the local generation of Ang II in the cardiovascular tissues.[7,8] However, possible involvement of other chymotrypsin-like enzymes such as cathepsin G or elastase could not be ruled out because mast cells or leukocytes are often observed in the vascular tissues under pathological conditions.[9,10] So, specific inhibitors of chymase will be useful as a tool to elucidate the physiological and/or pathological role of chymase and maybe as potential therapeutic agents.[11,12]

Chloromethyl ketone derivatives of amino acids such as Z-PheCH_2Cl (ZPCK, **1**)[13] and Tos-PheCH_2Cl (TPCK, **2**)[14] are classical serine protease inhibitors. The action of these agents is demonstrated to be attributed to the irreversible alkylation of imidazole nitrogen of the His residue at the active site of the enzymes.[15] Although the rate of inactivation is slow, irreversible and complete inhibition of the protease are expected. Therefore, these inhibitors should be useful to investigate the biological functions of chymase.

In the present study, we found that chloromethyl ketone derivatives **1** and **2** exhibited relatively selective inhibition against human chymase (h-chymase) (Table 1). Accordingly, to develop more potent and selective inhibitors of h-chymase, we synthesized a number of Z-PheCH_2Cl derivatives and the structure–activity relationship (SAR) study was performed. To assess the selectivity of each compound, inhibitory activity against h-chymase was compared with those of other serine proteases including bovine pancreatic α-chymotrypsin (α-CT), porcine pancreatic elastase (PPE), human leukocyte cathepsin G (h-CG), and porcine pancreatic trypsin (TRP). From this study, we found that compound **21** was a potent chymase inhibitor with no inhibitory activity against human cathepsin G, another physiological candidate responsible for the angiotensin II generation.[9,10]



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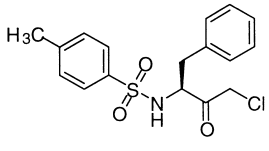

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Results and Discussion

Among all the protease inhibitors examined, we found that Z-PheCH_2Cl (**1**) and Tos-PheCH_2Cl (**2**), exhibited relatively selective inhibition against recombinant h-chymase[16] (Table 1). Particularly, **1** was about 8 times more potent ($IC_{50} = 0.40$ μM) than **2** ($IC_{50} = 3.3$ μM) and was about 40 and 60 times more selective towards h-chymase, when compared with the activities towards

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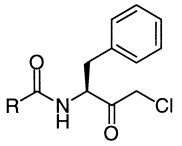
α-CT and h-CG, respectively, both of which belong to the chymotrypsin-like serine protease family as chymase. Therefore, we synthesized a series of compound **1** derivatives focused on its benzyloxycarbonyl (Z) portion, which can recognize the S2 subsite, responsible for enhancing the selectivity and to increase the activity for h-chymase inhibition.

In the first SAR study, a series of phenylalkylcarbonyl groups were introduced into H-PheCH_2Cl to investigate

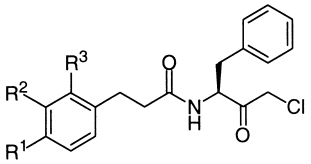
Table 1. Inhibition of serine proteases by derivatives of Z-PheCH_2Cl



Compd	R	IC_{50} (μM)[a]					Selectivity	
		h-chymase	α-CT	h-CG	PPE	TRP	α-CT / h-chymase	h-CG / h-chymase
1	benzyloxy (Z-PheCH_2Cl)	0.40	17	24	130	210	43	60
2	na[b] (Tos-PheCH_2Cl)	3.3	140	110	48	200	42	33
3	Ph	1.3	9.3	32	340	240	7	25
4	Bn	4.7	20	12	120	>1000	4	3
5	2-phenethyl	0.71	37	63	150	>1000	52	89
6	*trans*-styryl	15	8.7	17	26	210	<1	1
7	3-phenylpropyl	2.5	26	42	110	690	10	17
8	4-phenylbutyl	1.2	18	12	130	>1000	15	10

[a]The IC_{50} values of chymostatin for chymase, α-chymotrypsin and cathepsin G, elastatinal for elastase and leupeptin for trypsin are 0.44, 0.02, 0.025, 0.22 and 0.44 μM, respectively.[17]
[b]na = not applicable.

Table 2. Substitutions at the phenyl ring in compound **5**



Compd	R^1	R^2	R^3	IC_{50} (μM)[a]					Selectivity	
				h-chymase	α-CT	h-CG	PPE	TRP	α-CT / h-chymase	h-CG / h-chymase
5	H	H	H	0.40	17	24	130	210	43	60
9	Me	H	H	0.30	97	80	90	260	320	270
10	H	Me	H	0.43	11	25	130	290	26	58
11	H	H	Me	0.78	12	18	410	600	15	23
12	Et	H	H	1.4	37	35	85	120	26	25
13	isoPr	H	H	3.1	39	65	15	4	13	11
14	MeO	H	H	2.2	2.2	9.3	410	>1000	1	4
15	H	MeO	H	0.73	85	210	600	>1000	120	290
16	H	H	MeO	2.1	2.7	11	85	34	1	5
17	MeO	MeO	H	2.2	51	140	>1000	>1000	23	64
18	H	MeO	MeO	0.89	2.2	36	>1000	>1000	2	40
19	F	H	H	2.4	100	130	240	>1000	42	54
20	H	F	H	1.2	44	890	280	>1000	37	740
21	H	H	F	0.36	34	>1000	310	>1000	94	>2800
22	Cl	H	H	1.9	18	30	42	>1000	9	16
23	H	Cl	H	0.89	23	23	80	44	26	26
24	H	H	Cl	0.60	97	25	39	85	160	42
25	Cl	Cl	H	3.7	4.2	8.1	80	28	1	2
26	H	Cl	Cl	0.40	10	44	32	97	25	110
27	OH	H	H	2.7	14	91	>1000	>1000	5	34
28	H	OH	H	1.8	5.2	58	>1000	75	3	32
29	CF_3	H	H	2.5	17	32	13	30	7	13
30	H	CF_3	H	1.9	20	24	37	34	11	13

[a]See Table 1.

the optimal location of the phenyl ring in the Z group (compounds **3**–**8**). A carbamate structure in **1** was changed to an amide structure because of the availability of the units used in the modification. Amide bond formation with HCl·H–PheCH_2Cl was carried out by using the corresponding acylchloride in the presence of Et_3N in DMF, and the crude products were purified by silica gel column chromatography. The IC_{50} values[18] of the synthesized derivatives of **1** against several proteases are summarized in Table 1. The selective inhibition with the anti-chymase activity similar to **1** was obtained in compound **5**, which has the same atom numbers between the phenyl and carbonyl groups as **1**. The other compounds (**3**, **4**, **6**–**8**) with different distance between two functional groups showed reduced selectivity. These results indicate that a carbamate oxygen in **1** is not important and it is also suggested that the phenyl group in **5** is located at the appropriate position for the selective inhibition of h-chymase. A *trans*-styryl group was also introduced in compound **6**, which has the same atom number as **5**. This compound showed weak activity and lost selectivity, suggesting that an active conformation of **5** is not *trans*-structure at this position.

In the next SAR study, we introduced a series of substituting groups, both electron-donating groups (methyl, methoxy and hydroxyl groups) and electron-withdrawing groups (halogen atoms and trifluoromethyl group), onto the phenyl ring in compound **5** (Table 2). The introduction of various substituting groups resulted in marked variation in the anti-protease activity for all the enzymes, however, in the case of h-chymase, the variation was small with IC_{50} values ranging from 0.3 to 3.7 μM. This result suggests that recognition of the P2 portion by the S2 subsite would not be so definite in h-chymase, especially regarding the substitution on the phenyl ring. On the contrary, the substitutions have drastic effect on the inhibitory activities against other proteases, resulting in an increase in the selectivity towards h-chymase. Compound **9** containing a *p*-methyl substitution was about 300 times more selective towards h-chymase as compared with other chymotrypsin-like enzymes. Moreover, compound **21**,[19] which has a fluorine atom at the *o*-position, exhibited anti-chymase activity similar to **1**, with no inhibitory activity against human cathepsin G at 1 mM.

In conclusion, we have developed selective human chymase inhibitors, which are derivatives of a classical chloromethyl ketone derivative, Z-PheCH_2Cl. Compound **21** was found to be a potent chymase inhibitor with no inhibitory activity against human leukocyte cathepsin G. Therefore, it is expected that this compound could be very useful for probing the physiological and pathological roles of chymase and the molecular mechanisms responsible for the local generation of angiotensin II.

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18. Enzyme assay: The inhibitory effects of each compound on the enzymatic activities of five serine proteases were evaluated using the purified enzymes and the chromogenic substrates. Enzymes and those substrates used here were as follows, *N*-succinyl-Ala-Ala-Pro-Phe-pNA (1.5 mM) for human recombinant chymase and bovine pancreatic α-chymotrypsin (Sigma Type I-S; 47 ng/mL); *N*-succinyl-Ala-Ala-Pro-Phe-pNA (3 mM) for human leukocyte cathepsin G (Elastin Products Co., Inc.; 2.5 U/mL); *N*-succinyl-Ala-Ala-Ala-pNA (1 mM) for porcine pancreatic elastase (Sigma Type III; 0.8 mg/mL); Bz-L-Arg-pNA (1 mM) for porcine pancreatic trypsin (Wako Pure Chemicals; 1 U/mL). All experiments were carried out in 50 mM Hepes buffer (pH 7.4) containing 0.1 mM NaCl, except for the buffer used for chymase, which contained 50 mM Tris–HCl (pH 8.0) and 1 M KCl.
19. Compound **21**: white solid; mp 110–112 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.48 (t, J = 7.6 Hz, 2H), 2.95 (t, J = 7.6 Hz, 2H), 3.01 (dd, J = 5.1, 6.8 Hz, 2H), 3.89 (d, J = 16.2 Hz, 1H), 4.07 (d, J = 16.2 Hz, 1H), 4.92 (dd, J = 6.9, 14.2 Hz, 1H), 5.94 (d, J = 6.9 Hz, 1H), 6.96–7.11 (m, 4H), 7.13–7.34 (m, 5H); HRMS (FAB) calcd for $C_{19}H_{20}ClFNO_2$ $(M+H)^+$ 348.1167, found 348.1164. Anal. calcd for $C_{19}H_{19}ClFNO_2$: C, 65.61; H, 5.51; N, 4.03. Found: C, 65.41; H, 5.53; N, 3.89.