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Development of 6-benzyl substituted 4-aminocarbonyl-1,4-diazepane-2,5-diones as orally active human chymase inhibitors

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Abstract—A novel series of 6-benzyl substituted 4-aminocarbonyl-1,4-diazepane-2,5-diones was designed, synthesized, and evaluated as human chymase inhibitors. From this series, we identified several compounds which were effective, via oral administration, in a mouse model of chronic dermatitis.

In the preceding paper,[1] we reported 6-benzyl substituted 4-arylsulfonyl-1,4-diazepane-2,5-diones as a novel scaffold for human chymase inhibitors, and the inhibitor **1** (Fig. 1) and its analogues exhibited potent and selective inhibition of human chymase. However, in the course of our investigation into the application of these inhibitors as therapeutic agents, we found that **1** was relatively unstable in aqueous solution at neutral pH ($t_{1/2}$, 1.1 h, Table 1) and was hydrolyzed at the 5-position amide of the 1,4-diazepane ring, to ring-opening products. It seems essential to enhance the compound stability in order for it to be a drug candidate, since such a physicochemical property may hamper pharmaceutical development. In this report, we describe efforts to identify potent, stable, and orally active human chymase inhibitors, the 4-aminocarbonyl-1,4-diazepane-2,5-dione derivatives (Schemes 1 and 2).

From a structural viewpoint of compound **1**, chymase may be converted to an inactivated form, the acylated enzyme, formed by attack from the activated hydroxyl group of the catalytic Ser^{195} on the lactam carbonyl at the 5-position of **1**. It is likely that the reactivity of the lactam carbonyl at the 5-position, which is activated by the electron-withdrawing arylsulfonyl group at the 4-position, determines the ability for acylation of the active site serine residue, as well as instability in aqueous media. Based on the above considerations, we attempted to reduce the reactivity of the lactam carbonyl at the 5-position by changing the substituent at the 4-position to a less electron-withdrawing group. In the case of pyrrolidine-5,5-*trans*-lactam-based cytomegalovirus protease inhibitors[2] and azetidinone-based tryptase inhibitors,[3] the substituents on the lactam nitrogen affected the stability of the compounds, with the following order: CONHMe ≫ COMe > SO_2Me. Therefore, we selected the aminocarbonyl group as a less electron-withdrawing group and synthesized related compounds (Fig. 1).

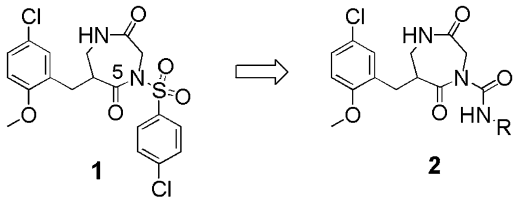

Figure 1. Replacement of electron-withdrawing group on the 4-position of the 1,4-diazepane-2,5-dione scaffold.

4-Aminocarbonyl-1,4-diazepane-2,5-dione-containing chymase inhibitors **14**–**29** listed in Tables 1 and 2 were

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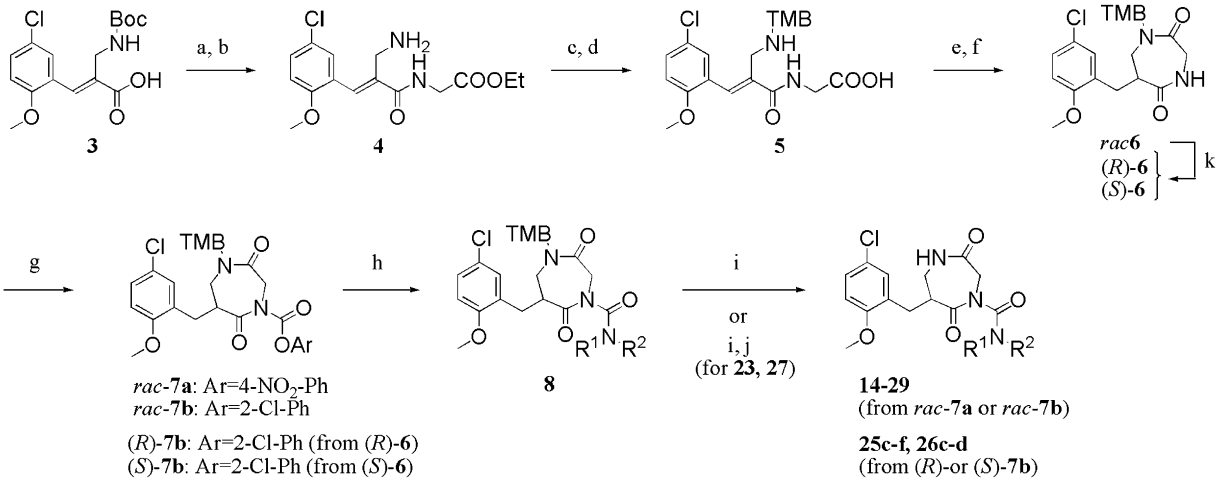
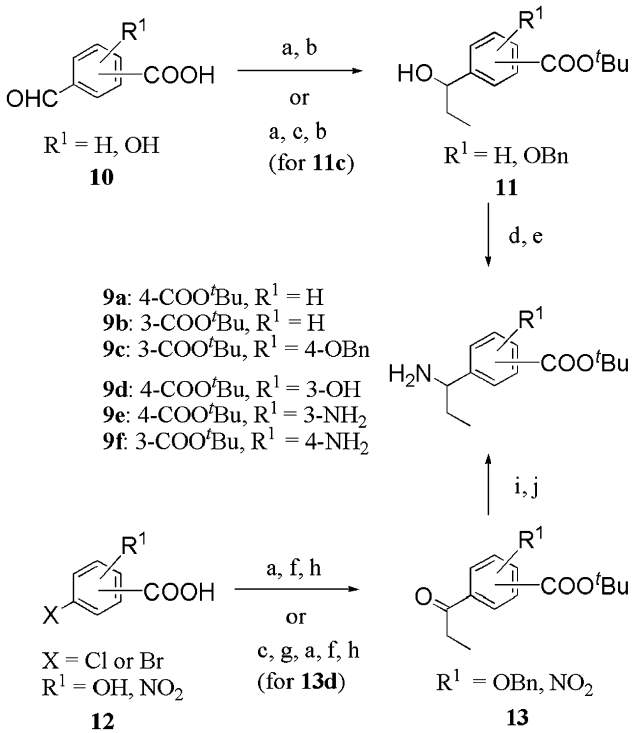


Scheme 1. Reagents and conditions: (a) glycine ethyl ester HCl, EDCI HCl, HOBt, Et_3N, CH_2Cl_2, rt, 2 h; (b) 4 M HCl/EtOAc, rt, 40 min; (c) 2,4,6-$(MeO)_3PhCHO$, $NaBH(OAc)_3$, THF, rt, 1.5 h; (d) aq NaOH, MeOH, 60 °C, 2.5 h; (e) EDCI HCl, HOBt, Et_3N, DMF, CH_2Cl_2, rt, 18 h; (f) H_2, Pt(sulfide)–C, THF, rt, 18 h; (g) BuLi, THF, −78 °C, 20 min then 4-NO_2PhOCOCl (for **7a**) or 2-ClPhOCOCl (for **7b**), −78 °C, 1 h; (h) amines, Et_3N, DMAP, DMF, 0 °C, 18 h; (i) 1 M HCl/AcOH, rt, 18 h; (j) trimethylsilyldiazomethane, EtOAc, MeOH, rt, 10 min; (k) optical resolution using chiral HPLC (CHIRALCEL OD).

prepared from β-amino acid **3**[1] via the racemic 7-membered lactam *rac*-**6** as a common intermediate (Scheme 1). Condensation of **3** with glycine ethyl ester hydrochloride, followed by deprotection of the Boc group, provided amine **4**. Introduction of the 2,4,6-trimethoxybenzyl group (TMB) to **4** by reductive alkylation, and subsequent hydrolysis of the ester moiety, gave amino acid **5**. Lactamization of **5**, followed by catalytic hydrogenation, afforded the 7-membered lactam *rac*-**6**. Then, *rac*-**6** was converted to the carbamate *rac*-**7**[4] by sequential treatment with *n*-butyllithium, then 4-nitrophenylchloroformate or 2-chlorophenylchloroformate. Coupling reactions of *rac*-**7** with amines furnished compounds **8**, which were deprotected to yield compounds **14**–**29**, except for **23** and **27**. Compounds **23** and **27** were prepared by esterification of the corresponding carboxylic acids **22** and **26**, respectively, with trimethylsilyldiazomethane. For compounds **22**–**29**, the diastereomers derived from the stereogenic centers of the 6-position of the diazepane ring and the amine moiety could be separated by chromatography at the purification step of **8**. The optically active compounds **25c**–**f** and **26c**,**d** were synthesized in a similar manner from optically pure 7-membered lactams (*R*)-**6** or (*S*)-**6**, which were prepared by optical resolution of *rac*-**6** using a chiral HPLC column (CHIRALCEL OD).

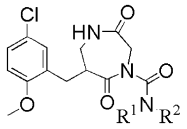
Phenylpropylamines **9a**–**c** for the synthesis of **22**, **26**, and **28** were prepared from aldehydes **10**, which were converted to secondary alcohols **11** by sequential manipulation (protection and Grignard reaction) (Scheme 2). Mitsunobu reaction of **11** using phthalimide, followed by deprotection, afforded **9a**–**c**. Other phenylpropylamine derivatives **9d**–**f** used for the synthesis of **24**, **25**, and **29** were prepared from halobenzoic acid derivatives **12**. Palladium-catalyzed arylation of nitropropane[5] with suitably protected **12** afforded α-arylnitropropanes, which were converted to propiophenones **13** by a Nef reaction. A two-step sequence of reactions (oxime formation and reduction) yielded amines **9d**–**f**.

Table 1 shows the chymase inhibitory activity and stability[6] in aqueous solution at pH 7.0 of compounds **14**–**21**. As expected, the stability of these compounds was increased compared with compound **1**. Among these, benzylaminocarbonyl derivative **14** showed



Scheme 2. Reagents and conditions: (a) *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea, *t*-BuOH, CH_2Cl_2, rt, 16 h; (b) EtMgBr, CuI, Et_2O, −23 °C, 30 min; (c) BnBr, K_2CO_3, DMF, rt, 2 h; (d) phthalimide, DEAD, PPh_3, THF, rt, 18 h; (e) hydrazine hydrate, MeOH, reflux, 1 h; (f) nitropropane, $Pd_2(dba)_3$, 2-(di-*tert*-butylphosphinyl)-2′-methylbiphenyl, K_3PO_4, DME, 100 °C, 24 h; (g) aq NaOH, MeOH, rt, 16 h; (h) KO *t*-Bu, 1,4-dioxane, then 1 M aq HCl; (i) hydroxylamine HCl, NaOAc, EtOH, reflux, 3 h; (j) H_2, Pd–C, EtOH, rt, 2–8 h.

Table 1. Inhibitory activity against recombinant human chymase and stability in aqueous solution of compounds **1** and **14**–**21**



Compound	R^1	R^2	IC_{50}[a] (μ M)	Stability[b] ($t_{1/2}$)
1[c]			0.034	1.1
14[c]	H	CH_2Ph	4.9	6.8
15[c]	Me	CH_2Ph	>100	>48
16[c]	H	CH_2CH_2Ph	44.5	12.9
17[d]	H	CH(Me)Ph	5.3	6.2
18[d]	H	CH(Et)Ph	2.8	6.3
19[d]	H	CH(*i*-Pr)Ph	3.0	8.0
20[d]	H	CH(*n*-Pr)Ph	69.3	7.6
21[c]	H	$CHPh_2$	>100	16.5

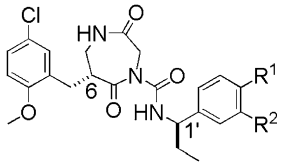
[a] For details of the assay conditions, see Ref. 1.
[b] For details of the assay conditions, see Ref. 6.
[c] Racemates.
[d] Mixture of diastereomers.

moderate activity, but *N*-methylation at the urea moiety (**15**) markedly diminished the activity. These results indicate that an intramolecular hydrogen bond between urea NH and carbonyl oxygen at the 5-position is critical to fix the conformation of inhibitors to interact with enzyme. Introduction of an alkyl group at the benzylic position of compound **14** resulted in retention or slight enhancement of the inhibitory activity (**17**–**19**), whereas substitution with a longer alkyl (**20**) or phenyl group (**21**), or chain elongation (**16**), decreased the activity. These results were confirmed by computer-assisted docking studies (vide infra) that indicated that the benzylaminocarbonyl moiety of **14** was situated around the S1′ and S2′ regions of the enzyme, and the substituent at the benzylic position interacted with a narrow and hydrophobic S1′ pocket. We next attempted to introduce the substituents on the phenyl ring of compound **18**, with the intention of making an interaction with prime site amino acids such as Lys^{40}, Phe^{41}, and Arg^{143} (Table 2). To elucidate the influence of the relative configuration derived from the two chiral centers, the 6-position of the diazepane ring and the 1′-position of the amine moiety, two possible racemic stereoisomers were synthesized and evaluated.[7] As shown in Table 2, (6*R**,1′*R**)-isomers were generally more potent than the corresponding (6*S**,1′*R**)-isomers. In the series of (6*R**,1′*R**)-isomers (left part of Table 2), installation of a carboxyl group at the *para*- (**22a**) or *meta*-position (**26a**) of the phenyl ring resulted in an increase in the inhibitory activity, but the corresponding methyl esters (**23a**, **27a**) were less potent. These results indicated that the carboxyl group may have been involved in the ionic interaction with Arg^{143} and/or Lys^{40} located at the prime site region. In contrast to our previous findings with 3-phenylsulfonylquinazoline-2,4-diones,[8] introduction of an additional substituent, such as a hydroxyl or amino group to **22a** or **26a**, did not further improve the potency (cf. **24a**, **25a**, **28a**, and **29a**). In the case of (6*S**,1′*R**)-isomers (right part of Table 2), only compound **25b**, which has a 3-amino and a 4-carboxyl group, showed high inhibitory activity. To examine the effects of the absolute configurations of the stereogenic centers of compounds **25a**, **25b**, and **26a** on inhibitory activity, we synthesized and evaluated the optically active compounds **25c**–**f** and **26c**,**d** (Table 3). In all cases, the (1′*R*)-isomers **25c**, **25e**, and **26c** were found to be more potent than the corresponding (1′*S*)-isomers. Furthermore, all the active enantiomers **25c**, **25e**, and **26c** showed high stability in aqueous solution (Table 3), and selectivity against other serine proteases, including chymotrypsin and chymotrypsin-type protease cathepsin G (Table 4).

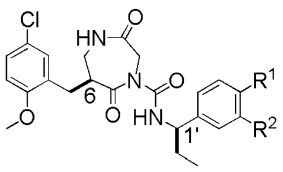
We investigated the in vivo activity of **25c**, **25e**, and **26c** in a mouse model of chronic dermatitis,[9] in which the enzymatic activity of chymase is involved in the development of skin edema. As shown in Table 5, oral administration of each compound at a dose of 10 mg/kg/day significantly inhibited the dermatitis.

Table 2. Inhibitory activity against recombinant human chymase of compounds **22**–**29**

(6*R**, 1′*R**)- configuration[a]



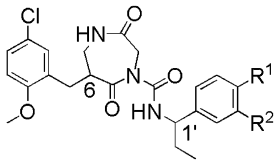
(6*S**, 1′*R**)- configuration[a]



Compound[a]	R^1	R^2	IC_{50}[b] (μM)	Compound[a]	R^1	R^2	IC_{50}[b] (μM)
22a	COOH	H	0.47	**22b**	COOH	H	5.0
23a	COOMe	H	2.5	**23b**	COOMe	H	14.2
24a	COOH	OH	0.46	**24b**	COOH	OH	1.8
25a	COOH	NH_2	0.5	**25b**	COOH	NH_2	0.34
26a	H	COOH	0.54	**26b**	H	COOH	4.3
27a	H	COOMe	2.6	**27b**	H	COOMe	51.4
28a	OH	COOH	0.41	**28b**	OH	COOH	4.1
29a	NH_2	COOH	0.63	**29b**	NH_2	COOH	6.1

[a] All compounds are racemates, and relative stereochemistry is represented.
[b] For details of the assay conditions, see Ref. 1.

Table 3. Inhibitory activity against recombinant human chymase and stability in aqueous solution of optically active compounds **25c–f** and **26c,d**



Compound	R^1	R^2	Configuration		IC_{50}[a] (μM)	Stability[b] ($t_{1/2}$)
			6-	1′-		
25c	COOH	NH_2	*R*	*R*	0.30	6.7
25d	COOH	NH_2	*S*	*S*	>10	—[c]
25e	COOH	NH_2	*S*	*R*	0.17	6.1
25f	COOH	NH_2	*R*	*S*	>10	—[c]
26c	H	COOH	*R*	*R*	0.24	7.4
26d	H	COOH	*S*	*S*	>10	—[c]

[a] For details of the assay conditions, see Ref. 1.
[b] For details of the assay conditions, see Ref. 6.
[c] Not measured.

Table 4. IC_{50} values[a] of inhibition of human chymase and other serine proteinases for compounds **25c**, **25e**, and **26c**

Enzyme	Compound		
	25c	**25e**	**26c**
Human chymase	0.30	0.17	0.24
Bovine α-chymotrypsin	>100	23.0	56.0
Human cathepsin G	16.0	28.0	8.0
Bovine trypsin	>100	>100	>100
Human elastase	>100	>100	>100

[a] For details of the assay conditions, see Ref. 1.

Table 5. Effect of compounds **25c**, **25e**, and **26c** on ear edema in a mouse model of chronic dermatitis[a]

Compound	% inhibition[d]
25c[b]	29.7%**
25e[b]	35.8%**
26c[b]	22.9%*
Prednisolone[c]	54.6%**

[a] See Ref. 9 and 10 for details.
[b] Each compound was orally administered at 10 mg/kg once a day during the experimental period.
[c] Prednisolone was administered intraperitoneally at 10 mg/kg once a day during the experimental period.
[d] Inhibition ratio of the ear thickness at 24 h after the sixth painting of DNFB.
* $P < 0.05$ versus vehicle (Student's *t* test).
** $P < 0.01$.

A docking structure[11] of compound **26c** in the active site of human chymase (Fig. 2) indicated that: (1) the 5-chloro-2-methoxybenzyl group occupied the S1 pocket; (2) the 1,4-diazepane ring was located in the proper position at which the amide-NH at the 1-position formed a hydrogen bond with the Ser^{214} backbone amide carbonyl; (3) the phenylpropylamine moiety was located in the prime site region of the enzyme, and the ethyl substituent occupied the hydrophobic S1′ pocket constructed by Lys^{40}, Cys^{42}, and Cys^{58} side chains; and (4) the carboxyl group on the phenyl ring electrostatically interacted with the Arg^{143} side chain, and this ionic interaction may have accounted for the increase in the inhibitory activity of the benzoic acid derivatives. To

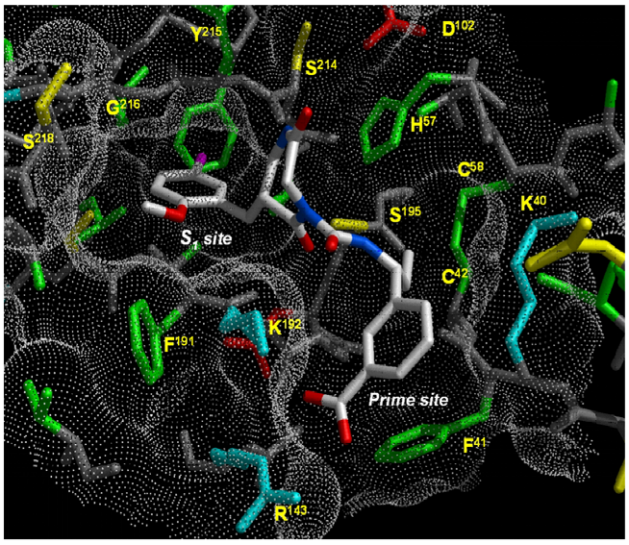


Figure 2. Docking structure of compound **26c** in the active site of human chymase.

clarify the binding mode of this class of inhibitors, further studies including the X-ray crystal structure analysis of chymase with inhibitors are currently under way and the results will be reported in the near future.

In conclusion, we have reported 4-aminocarbonyl-1,4-diazepane-2,5-diones as being potent, stable and, orally active human chymase inhibitors. Compounds **25c**, **25e**, and **26c** were orally active in a mouse model of chronic dermatitis, in which chymase activity may be involved. These results indicate that 4-aminocarbonyl-1,4-diazepane-2,5-dione is a promising scaffold for chymase inhibitors that are possible therapeutic candidates for diseases such as atopic dermatitis.

Acknowledgments

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References and notes

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6. Each compound was incubated at 37 °C in 0.1 M phosphate buffer (pH 7.0) at a concentration of 10 μM for the following times: 0, 4, and 8 h. The ratio of the remaining compounds was measured by HPLC analysis.
7. The relative configuration of **26b** was determined by X-ray crystal structure analysis of the corresponding intermediate **8**. As for the other compounds, the stereochemistry was tentatively determined by comparison of the chromatographic behavior of the separated diastereomers with that of **26a** and **26b**. Furthermore, we confirmed the relative configuration of compound **25e** by X-ray crystal structure analysis.
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