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PEPTIDYL HUMAN HEART CHYMASE INHIBITORS. 1. SYNTHESIS AND INHIBITORY ACTIVITY OF DIFLUOROMETHYLENE KETONE DERIVATIVES BEARING P' BINDING SUBSITES

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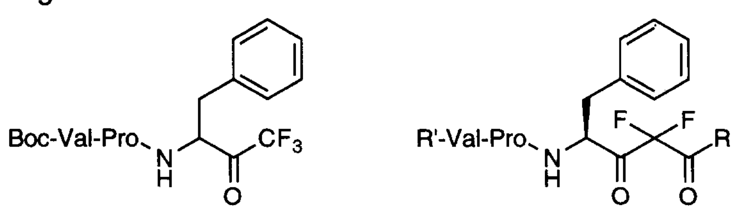
Abstract: Peptidyl difluoromethylene ketone derivatives were designed to take advantage of probable additional interactions with the S' subsite of human heart chymase. They showed potent inhibitory activities against human heart chymase and were more efficient than bovine chymotrypsin.

Human heart chymase (HHC) is a chymotrypsin-like serine protease, which was identified in the left ventricular tissues of the human heart and characterized by Urata.[1] It is a highly efficient and specific enzyme which catalyzes the conversion of angiotensin I (Ang I) to angiotensin II (Ang II).[2] Ang II has a variety of physiological roles in cardiovascular homeostasis.[3] Specific inhibitors of Ang I converting enzyme (ACE) are widely used in the treatment of hypertension[4] and congestive heart failure.[5] Okunishi reported that Ang II formation in human arteries depends more on chymase than on ACE.[6] Development of specific inhibitors of HHC, therefore, may contribute to studies of the physiological functions of this protease and better treatment of cardiovascular diseases.

One approach to design serine protease inhibitors has been replacement of the scissile amide bond by an electron-deficient carbonyl group such as α-diketone, trifluoromethyl ketone (TFMK), difluoromethylene ketone (DFMK), or α-ketoheterocycle.[7] These derivatives are believed to form a metastable hemiketal with the catalytic center Ser195, which resembles the tetrahedral intermediate in the reaction pathway for enzyme-substrate hydrolysis. Initially, we synthesized TFMK inhibitor **1** which favorably interacts with S subsites of HHC through Val-Pro-Phe at the P positions as confirmed by kinetic studies with HHC using peptide 4-nitroanilides[8] and hormones as substrates.[9] Although peptidyl TFMK **1** showed moderate chymase inhibitory activity as expected, it lacked selectivity against the closely related serine protease bovine α-chymotrypsin (BCT).

Figure 1



1: Chymase Ki = 56nM
Chymotrypsin Ki = 113nM

2

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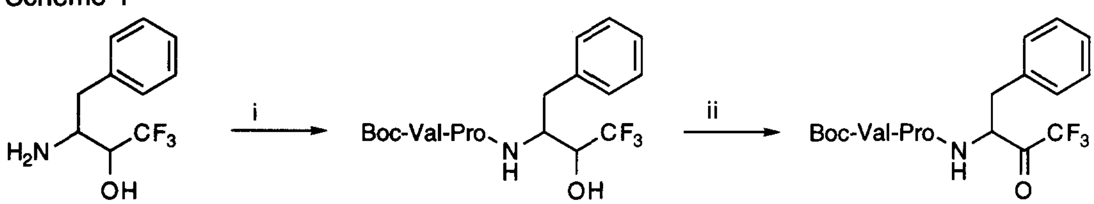
Kinoshita proposed that the unusually high substrate specificity of HHC for Ang I is due to the unique conformation of the S' subsite of HHC.[9] We hypothesized that inhibitors which can interact with the S' subsite would increase affinity for HHC and specificity against other chymotrypsin-type serine proteases. Based on this hypothesis, we designed and synthesized peptidyl DFMK derivatives **2** to take advantage of the probable additional interactions with the S' subsite of HHC. In this report, we describe the synthesis and structure-activity relationships (SAR) of this series of DFMK derivatives. We found that either hydrophobic or ionic interactions at this site are necessary for potent HHC inhibitory activity.

Chemistry[10]

Preparation of the peptidyl TFMK **1** is shown in Scheme 1. Amino trifluoro alcohol **3**[11] was coupled to Boc-Val-Pro-OH using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP) in the presence of triethylamine (TEA) to give the alcohol **4**. Subsequent Dess-Martin oxidation afforded the target TFMK **1**. Methods for preparation of the C-terminal and N-terminal modified DFMK derivatives are shown in Scheme 2. Removal of the Boc group from the known ester **5**[12] with hydrogen chloride in dioxane, followed by coupling to Boc-Val-Pro-OH, afforded the peptidyl ester **6**. The ester was hydrolyzed with 1 equiv of sodium hydroxide and the resulting salt **7** was condensed with various amines or amino acid benzylesters using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBT) to give the alcohol **8**.[12] Deprotection of **8**, followed by reaction with various acyl chlorides or sulfonyl chlorides led to the N-terminal modified alcohol **9**. After oxidation of alcohols **8** and **9** with Dess-Martin periodinane and trituration with ether, the DFMK derivatives **10-12** were obtained as solids with minor diastereomers due to rapid epimerization at the α-carbon of P1 phenylalanine. Without separation of the diastereomers, the DFMK benzyl esters **11** and **12** were hydrogenated to give the acids **13** and **14**, respectively. Starting with (3*S*,4*R*)-isomer **15** obtained from Boc-D-phenylalaninal, the corresponding D-isomer **16** of **10c** was similarly prepared.

Scheme 1[a]



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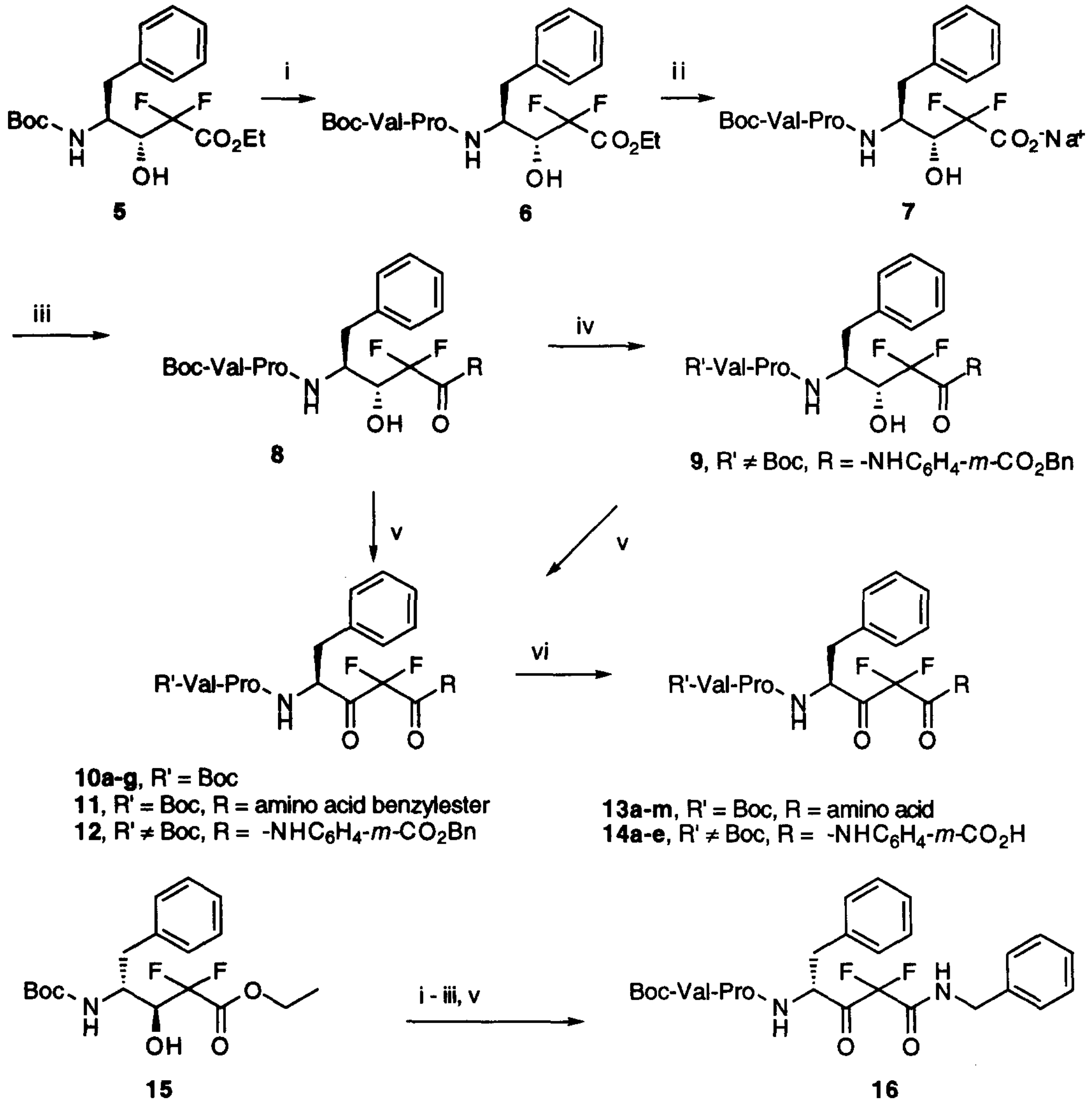
[a]Reagents: (i) Boc-Val-Pro-OH, BOP, TEA (81%); (ii) Dess-Martin periodinane (37%).

Scheme 2[a,b]



[a]Reagents: (i) a) HCl, dioxane, b) Boc-Val-Pro-OH, BOP, TEA (85 %); (ii) 1N NaOH (100 %); (iii) amine, EDCI, HOBt (62 %); (iv) a) HCl, dioxane, b) acyl chloride or sulfonyl chloride, TEA (85 %); (v) Dess-Martin periodinane (73 %); (vi) H_2, 10% Pd/C (66 %).
[b]The yields reported (%) are for the synthesis of **14e**.

Enzyme assay

HHC was kindly provided by Prof. Miyazaki and Dr. Shiota, Osaka Medical College. BCT was purchased from Sigma Chemical Co. (St Louis, MO). Enzymatic activity of HHC and BCT was regarded as the rate of hydrolysis of synthetic substrate, succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (Suc-AAPF-*p*NA) (Sigma Chemical Co.). The inhibitory effects of test compounds on HHC and BCT were assayed by the following methods. Briefly, the synthetic substrate (2.5 mM the final concentration) was added to assay buffer (20 mM Tris-HCl (pH 7.5) containing 2 M KCl) with five different concentrations of test compound and 10 % DMSO in H_2O as a control. Reaction was started by adding enzyme solution. The change of absorbance was measured at 405 nm with the subtraction at 650 nm after 2 hour incubation at 37 °C. The residual enzymatic activity was determined at each concentration of test compound followed by the plot from the equation of Easson and Stedman.[13] All Ki values were obtained from the equation: Ki = Ki' x Km / (Km + [S]), where Ki' is the observed Ki value of an appropriate inhibitor and [S] indicates the initial substrate concentration. Km values were determined from Lineweaver-Burk plots.

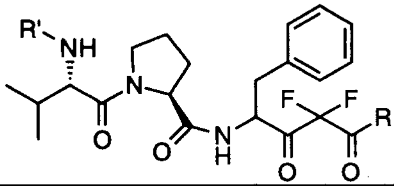
Results and Discussion

The enzyme inhibitory activities for HHC and BCT are summarized in Table 1. Many of the DFMK derivatives exhibited good inhibitory activity for HHC and high BCT/HHC selectivity compared with the prototypical TFMK derivative **1**, as we had hypothesized. The inhibitory activity for HHC increased by introducing simple methylamino and anilino groups (**10a** and **b**) into the plausible P' site. Extending the length of the methylene insert between the phenyl ring and amino group of **10b** improved inhibitory activity for HHC with a benzylamine substituted analog **10c** (Ki = 7.4±1.8 nM) being optimal among those examined. Replacement of benzylamino by N-methylbenzylamino group resulted in a 10-fold decrease in inhibitory activity for HHC (**10c** vs **10e**). The effect of stereochemistry at the P1 site was studied by comparing **10c** with the corresponding D-isomer **16**. The unnatural D-isomer **16** showed reductions in inhibitory activities for HHC and BCT of about 10-fold and 100-fold, respectively. Angelastro reported that epimerization at the P1 residue of pentafluoroethyl ketone derivatives progressed readily in solution in a pH- and temperature-dependent manner.[14] Although we did not determine the extent of epimerization of DFMK derivatives in our assay system, our results suggested that extensive epimerization may not have occurred, and that HHC may have a more flexible S1 recognition site than that of BCT.

Interestingly, with an glycine residue in place at the C-terminal substituent, **13a** (Ki = 13.1±1.9 nM, BCT/HHC = 200) showed potent inhibitory activity for HHC and considerable loss of activity for BCT compared with **1**. Extending the length of the alkyl chain in the glycine moiety resulted in slight loss of activity (**13a** vs **13b-c**), while the methylester derivative **10f** (Ki = 6.5±1.1 nM, BCT/HHC = 387) retained potency for both enzymes as in **13a**.

Systematic modifications of the P' position of the DFMK derivatives were also carried out by replacing the glycine residue (**13a**) with a variety of amino acid residues (**13d-j**) and carboxyanilino groups (**13k-m**). The markedly reduced potency of the Pro derivative **13e** as well as **10e** suggested that the N-H group of this amide bond is necessary for potent activity. We observed that not only the phenyl group but also the carboxylic acid group at P' position is important for the potency against HHC (*vide supra*). Interestingly, the existence of both groups in this position or additional carboxylic acid group had a highly detrimental effect on enzyme inhibition (**13f-j**). The *ortho*-aminobenzoic acid substituent (**13k**) also reduced the inhibitory potency, whereas the *meta* and *para* substituted isomers (**13l** and **m**) were as potent as **10c**. From these findings, we speculate that both position of the phenyl group and direction of the carboxylic acid have significant effects on potency of HHC inhibitory activity.

Table 1. Inhibitory Activity and Specificity of DFMK Derivatives.



No	R'	R	Inhibitory Activity Ki (nM)[a]		selectivity
			Chymase (HHC)	Chymotrypsin (BCT)	BCT (Ki)/HHC (Ki)
1	(trifluoromethyl ketone)		55.8±4.1	113±11	2
10a	Boc	NHMe	28.7±4.3	1070±48	37
10b	Boc	NHPh	16.3±5.3	70±10	4
10c[b]	Boc	NHCH$_2$Ph	7.4±1.8	172±5	23
16[c]	Boc	NHCH$_2$Ph	92.4±3.7	11900±2110	129
10d	Boc	NH(CH$_2$)$_2$Ph	18.3±0.9	2040±147	111
10e	Boc	N(Me)CH$_2$Ph	70.1±4.1	7550±771	108
13a	Boc	Gly-OH	13.1±1.9	2620±473	200
10f	Boc	Gly-OMe	6.5±1.1	2510±810	387
10g	Boc	Gly-OCH$_2$Ph	12.5±1.6	316±6	25
13b	Boc	NH(CH$_2$)$_2$CO$_2$H	36.4±7.6	2300±89	63
13c	Boc	NH(CH$_2$)$_3$CO$_2$H	24.0±5.9	4430±494	185
13d	Boc	Ala-OH	65.6±10.7	54900[d]	837
13e	Boc	Pro-OH	493±77.5	>100000	>203
13f	Boc	Phe-OH	30.1±11.1	1700±69	57
13g	Boc	(D)-Phe-OH	513±7.8	77400±37200	151
13h	Boc	Asp-OH	1550±77.9	>100000	>65
13i	Boc	Glu-OH	1010±126	>100000	>99
13j	Boc	NHCH(Ph)CO$_2$H[e]	1040±200	>100000	>96
13k	Boc	NHC$_6$H$_4$-o-CO$_2$H	3620±64.1	>100000	>28
13l	Boc	NHC$_6$H$_4$-m-CO$_2$H	5.6±1.9	364±27	65
13m	Boc	NHC$_6$H$_4$-p-CO$_2$H	6.8±0.7	200±6	29
14a	PhSO$_2$-	NHC$_6$H$_4$-m-CO$_2$H	6.3±1.2	554±64	88
14b	MeSO$_2$-	NHC$_6$H$_4$-m-CO$_2$H	16.5±2.2	207±15	13
14c	PhCO-	NHC$_6$H$_4$-m-CO$_2$H	3.1±1.1	275±12	88
14d	Ac-	NHC$_6$H$_4$-m-CO$_2$H	7.1±1.3	11±2	2
14e	Ph(CH$_2$)$_2$CO-	NHC$_6$H$_4$-m-CO$_2$H	1.3±0.4	19±1	15

[a] The values are means±SEM of three independent experiments (n = 3). [b] Ratio of epimers (*S*:*R*) at P1 stereocenter by HPLC is 98:2. [c] Ratio of epimers (*S*:*R*) at P1 stereocenter by HPLC is 3:97. [d] n = 2. [e] The absolute configuration at the stereocenter of substituent R is (*S*)-configuration.

Additional experiments were performed to explore the SAR for the P4 position by replacing the Boc group of **13l** with various acyl and sulfonyl groups. Compounds **14a-d** showed potent HHC inhibitory activity similarly to **13l**. The compound **14e** with a phenylpropionyl group was the most potent HHC inhibitor among the derivatives investigated (Ki = 1.3 nM); however, a simultaneous increase of potency for BCT reduced the selectivity relative to that of **13l** (**13l**; Ki = 5.6±1.9 nM, BCT/HHC = 65 vs **14e**; Ki = 1.3±0.4 nM, BCT/HHC = 15). This result suggested that suitable combination of P' and P4 residues is important for high selectivity.

In conclusion, we generated potent and specific peptidyl HHC inhibitors by modification of DFMK derivatives at the P' position. Our results also provided variable information on SAR for HHC inhibitors, and we are currently engaged in the design of non-peptide inhibitors.

Acknowledgments

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References and Notes

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10. All new compounds were characterized by spectroscopic (NMR, IR, low resolution MS) data. For a representative compound **10c**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.65 (t , J = 6.0 Hz, 1H), 8.48 (d, J = 7.1 Hz, 1H), 7.35-7.25 (m, 10H), 6.72 (d, J = 8.5 Hz, 1H), 4.98 (ddd, J = 8.6, 7.1, 3.8 Hz, 1H), 4.36 (m, 2H), 4.34 (m, 1H), 3.97 (m, 1H), 3.67 (m, 1H), 3.53 (m, 1H), 3.15 (dd, J = 14.4, 3.9 Hz, 1H), 2.81 (dd, J = 14.4, 8.9 Hz, 1H), 1.99 (m, 1H), 1.88 (m, 1H), 1.84 (m, 2H), 1.75 (m, 1H), 1.36 (s, 9H), 0.95-0.80 (m, 6H). IR (KBr) 3425, 3250, 2950, 1750, 1700, 1635, 1495, 1430, 1365, 1160 cm^{-1}. MS (SIMS) *m/z* 629 (MH^+).
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