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PEPTIDYL HUMAN HEART CHYMASE INHIBITORS. 2. DISCOVERY OF HIGHLY SELECTIVE DIFLUOROMETHYLENE KETONE DERIVATIVES WITH GLU AT P3 SITE

Masahiro Eda, Atsuyuki Ashimori,* Fumihiko Akahoshi, Takuya Yoshimura, Yoshihisa Inoue, Chikara Fukaya, Masahide Nakajima, Hajime Fukuyama, Teruaki Imada, Norifumi Nakamura.

Department of Medicinal Chemistry, Green Cross Research Laboratories, 2-25-1, Shodai-Ohtani, Hirakata, Osaka 573, Japan.

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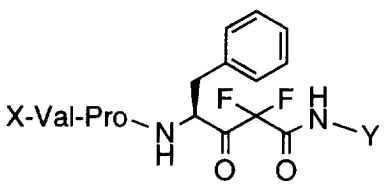
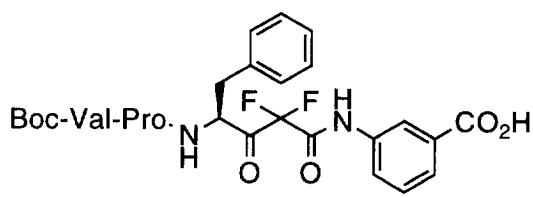
Abstract: Appropriate structural modification of the difluoromethylene ketone derivatives at both P3 and P' positions led us to the discovery of peptidyl human heart chymase inhibitor **12h** which shows potent activity with Ki = 6 nM and high selectivity against closely related serine protease bovine α-chymotrypsin (chymotrypsin Ki = >100 μM). Using the compound **12b**, a docking study with human heart chymase was carried out to presume probable interactions.

Human heart chymase (HHC) is a chymotrypsin-like serine protease that converts angiotensin I to angiotensin II.[1] Although the physiological role of this enzyme had not been fully elucidated, experimental data[2] suggested that it may be involved in various pathological states, particularly in cardiovascular diseases. Previously, we reported structure-activity relationships of difluoromethylene ketone (DFMK) derivatives exemplified by 1 (Figure 1), and that either a phenyl or a carboxylic acid group at the P' position is necessary for high binding affinity and selectivity for HHC against closely related serine protease bovine α-chymotrypsin (BCT). Compound **2** (GCC-AU0422), in particular, had a remarkable profile (Ki = 5.6 nM and BCT/HHC = 65) superior to the corresponding *p*- and *o*-carboxy derivatives. These results suggested that P' components of **2** interact well with the characteristic conformation of the S' subsite of HHC. Modification of the P4 position (X in compound **1**) improved affinity for HHC, but significantly increased inhibitory activity for BCT as well and consequently causing a loss of selectivity for HHC against BCT.

Figure 1



1



2: GCC-AU0442

3: Z-Ile-Glu-Pro-Phe-CO_2Me

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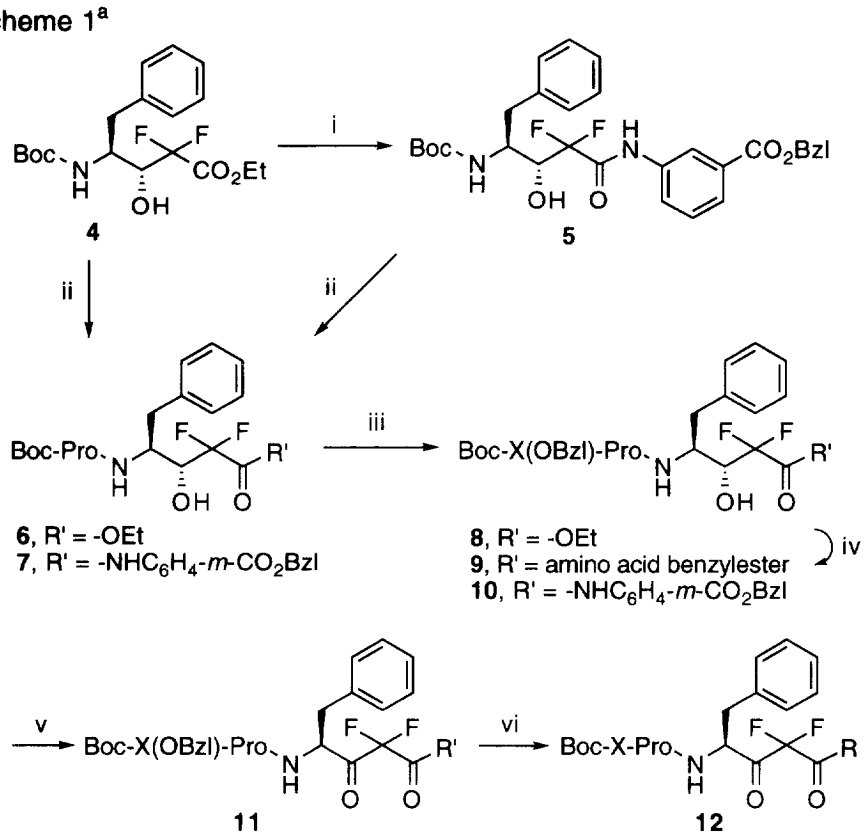
Bastos reported non-cleavable α-diketo peptide-inhibitor libraries to map the S and S' subsites of HHC.[3] He identified inhibitor **3** with Ki of 1 nM for HHC and 10 nM for BCT. This suggested that glutamic acid (Glu) at the P3 position may be more favorable than a valine residue (Val) for enzyme affinity. Accordingly, we synthesized analogs of **2** with an acidic amino acid (Glu or Asp) at the P3 position and further modified the P' structure of the Glu analog.

Here, we report the synthesis and HHC and BCT inhibitory activities of these analogs. Compound **12h** [Boc-Glu-Pro-PheCF$_2$CONH(4-carboxy-2-thienyl)] has been identified as a potent and highly selective HHC inhibitor. We also report docking and interaction studies of **12b** [Boc-Glu-Pro-PheCF$_2$CONH(3-carboxyphenyl)] with an HHC model.

Chemistry

Scheme 1[a]



[a]Reagents: (i) a) 1N NaOH, b) m-aminobenzoic acid, EDCI, HOBt (62 %); (ii) a) HCl, dioxane, b) Boc-Pro-OH, BOP, TEA (81 %); (iii) a) HCl, dioxane, b) Boc-X-OH, BOP, TEA (76 %); (iv) amine, BOP, HOBt, pyridine; (v) Dess-Martin Periodinane (90 %); (vi) H_2, 10% Pd/C (76 %).
[b]The yields reported (%) are for **12b**.

Preparations of the modified DFMK derivatives at P3 and P' positions are shown in Scheme 1. The known ester **4** was hydrolyzed with 1 equiv of sodium hydroxide, and the resulting salt was condensed with the *m*-aminobenzoic acid using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBT) to give the alcohol **5**.[4] Deprotection of **4** and **5** with hydrogen chloride in dioxane, followed by coupling to Boc-Pro-OH, afforded alcohols **6** and **7**, respectively. The protecting groups of **6** and **7** were similarly removed, and the obtained amine hydrochlorides were coupled to Boc protected benzyl glutamate and aspartate to give **8** and **10**, respectively. The alcohol **8** was hydrolyzed with 1 equiv of sodium hydroxide, and the resulting salt was condensed with various amino acid benzylesters, an amino carboxamide, or amino sulfonamides using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP) and HOBT in pyridine to give the alcohol **9**. Oxidation of alcohols **9**, **10** with Dess-Martin periodinane,[5] followed by catalytic hydrogenation and trituration with ether, afforded the DFMK derivatives **12a-i** as solids. All the inhibitors in Table 1 are racemic at the α-carbon of P1 phenylalanine and were not further separated.

Docking Study

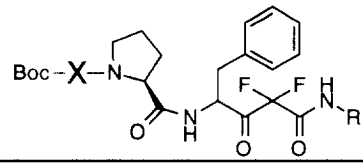
The docking study was performed as follows. The three-dimensional model of HHC was constructed based on the coordinate data of rat mast cell protease-II (RMCP II) in the Brookhaven Protein Data Bank[6] using the HOMOLOGY program (MSI, San Diego, California), because the sequence of RMCP-II is the most homologous to that of human. The model was energy-minimized using the DISCOVER program (MSI) with backbone-fixed restrictions. The three-dimensional model of compound **12b** was constructed based on standard bond angles and lengths and then energy-minimized using the DISCOVER. The resulting conformers were docked into the HHC model using SYBYL (Tripos, St.Louis) on an IRIS computer (Silicon Graphics Inc., Mountain View, California) to give a plausible model of HHC-inhibitor interactions.

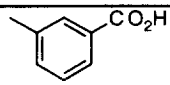
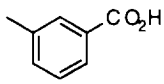
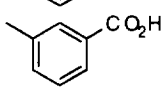
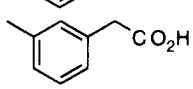
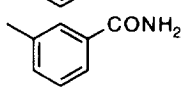
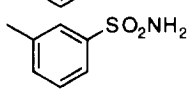
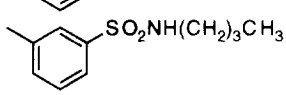
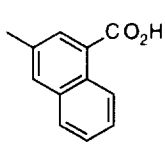
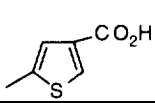
Results and Discussion

The enzyme inhibitory activities were measured by the previously described method, and the results are summarized in Table 1. Replacement of the Val in **2** with an Asp residue at the P3 position decreased HHC inhibitory activity (**12a**), while Glu derivative **12b** retained activity comparable to that of **2**. Both **12a** and **12b** significantly decreased inhibition of BCT, and accordingly BCT/HHC selectivity was increased. These results suggest that a Glu residue at the P3 site provides a favorable interaction with HHC as well as a Val residue (**2** and **12b**), which is not shared in the compound with an Asp residue (**12a**). The differences in activities among **2**, **12a** and **b** clearly indicates distinct structures between these enzymes.

Next, we modified the P' position of **12b** to determine whether other favorable interactions produced by the Glu residue influence the C-terminal interaction with enzymes. Changing the carboxyl group to carboxymethyl (**12c**), carbamoyl (**12d**), or aminosulfonyl groups (**12e-f**) did not improve HHC inhibitory activity. Replacement of the benzene ring by naphthalene (**12g**) or thiophene (**12h**) did not increase inhibitory activity for HHC. However, they showed considerable loss of activity for BCT. This series of structural modifications indicated that HHC prefers a carboxy function at the C-terminus and has a relatively loose space tolerable at least for the size of naphthalene, whereas BCT is far more sensitive to steric factors at this position. HHC and BCT inhibitory activities for the reference compound **3** measured in our assay system were comparable to those reported. Consequently, we found that the DFMK derivative **12h** with HHC inhibitory activity as potent as that of **3** also showed remarkable selectivity against BCT (>10000).

Table 1. Inhibitory Activity and Specificity of DFMK Derivatives and Reference Compound.



No	X	R	Inhibitory Activity Ki (nM)[a]		selectivity
			Chymase (HHC)	Chymotrypsin (BCT)	BCT (Ki)/HHC (Ki)
2	Val		5.6±1.9	364±27	65
12a	Asp		36.6±3.3	>100000	2732
12b	Glu		3.9±0.7	1300±49	332
12c	Glu		6.7±1.1	3950±504	590
12d	Glu		21.1±6.3	1220±86	58
12e	Glu		27.5±4.2	1030±41	38
12f	Glu		22.8±3.6	476±51	21
12g	Glu		14.2±1.2	92900[b]	6562
12h	Glu		6.0±0.7	>100000	>16667
3[c]	reference compound (see Figure1)		3.0±0.7	34±1	12

[a] The values are means±SEM of three independent experiments. [b] n = 2. [c] ref. 3.

We suggest two putative docking models of the compound **12b** with a three-dimensional model of HHC. The first model shown in Figure 2(a) indicates that the P3 amido N atom and carbonyl O atom form hydrogen bonds with Gly216 carbonyl O atom and amido N atom in an antiparallel manner, and that a hydrogen bond is present between the carbonyl O atom of Ser214 and amido N atom of the P1 residue. The P1 and P2 side chains make hydrophobic contacts in the S1 and S2 specificity pockets, respectively. The P3 side chain is exposed to the solvent. The activated carbonyl group of the inhibitor undergoes nucleophilic attack by the hydroxy oxygen of Ser195, and the resulting hemiketal oxygen is in the oxyanion hole and makes hydrogen bonds with the backbone amido N's of Ser195 and Gly193. We also propose triplicate favorable interactions of

the carboxyphenylamido group at the P' site consisting of (1) hydrogen bond of amido N atom with Phe41 carbonyl O atom, (2) aromatic-aromatic interaction with Phe41 side chain, and (3) electrostatic interaction of the terminal carboxylic acid with the side chain of Lys40. The lack of the third interaction owing to the absence of Lys40 in BCT may be responsible for increased selectivity to HHC against BCT. Essentially the same interaction would be observed in compound **2** (P3 = Val). Another possible model is shown in Figure 2(b), where the Glu side chain is flipped toward Ser218 forming a hydrogen bond and the Boc group could be in van der Waals contact with the side chain of Tyr215. The stabilizing effect thus obtained could overcome the energy increase caused by breaking of the hydrogen bond between carbonyl O atom of Gly216 and amido N atom of the P3 residue. Determination of the actual shape of the complex must await X-ray crystal structure analysis.

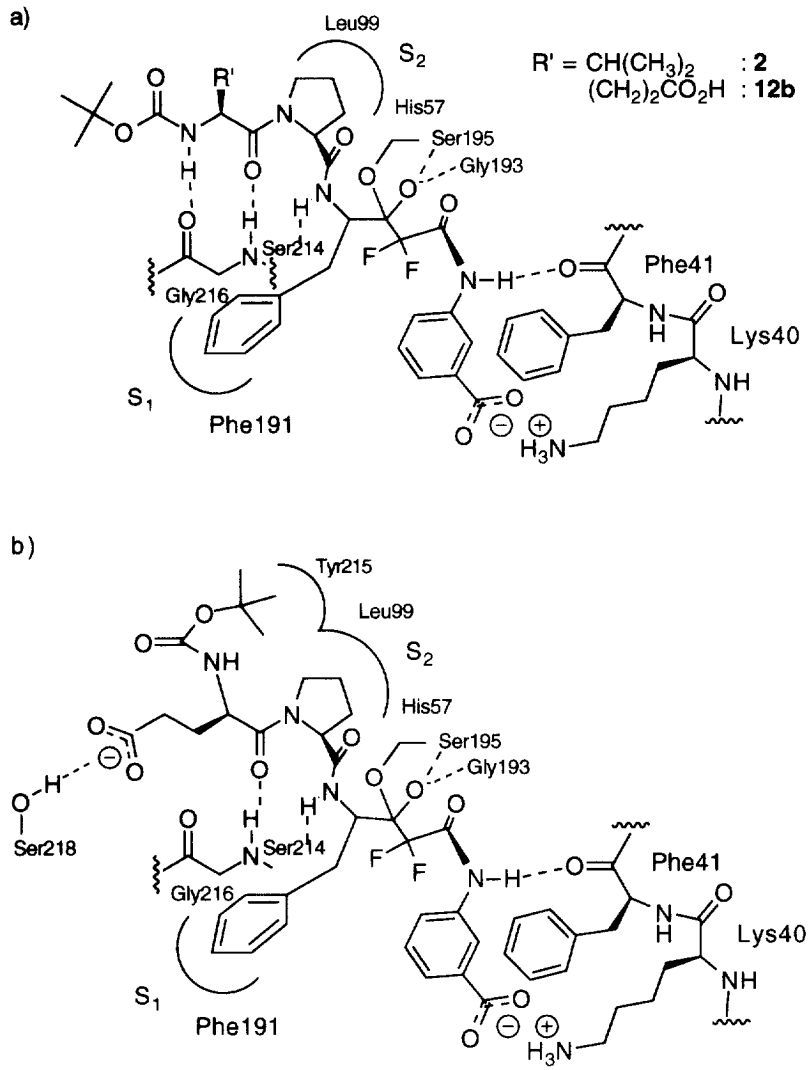


Figure 2. Schematic illustration of the key interactions of **2** and **12b** complexed to HHC based on the docking study.

In conclusion, by appropriate modification of the DFMK derivatives at both P3 and P' positions, we found peptidyl HHC inhibitor **12b** showing potent inhibitory activity and high selectivity against BCT. Docking study of **12b** with HHC suggested the possibility of unique interactions at P3 and P' subsites.

Acknowledgments

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References

1. Urata, H.; Kinoshita, A.; Misono, K. S.; Bumpus, F. M.; Husain, A. *J. Biol. Chem.* **1990**, *265*, 22348. Peach, M. *J. Phisiol. Rev.* **1997**, *57*, 313.
2. Shiota, N.; Okunishi, H.; Fukamizu, A.; Sakonjo, H.; Kikumori, M.; Nishimura, T.; Nakagawa, T.; Murakami, K.; Miyazaki, M. *FEBS Lett.* **1993**, *323*, 239.
3. Bastos, M.; Maeji, N. J.; Abeles, R. H. *Proc. Natl. Sci. USA* **1995**, *92*, 6738.
4. Thaisrivongs, S.; Pals, D.T.; Kati, W. M.; Turner, S.R.; Thomasco, L. M.; Watt, W. *J. Med. Chem.* **1986**, *29*, 2080.
5. Linderman, R. J.; Gravas, D. M. *J. Org. Chem.* **1989**, *54*, 661.
6. Remington, S. J.; Woodbury, R. G.; Reymonds, R. A.; Matthews, B. W.; Neurath, H. *Biochemistry* **1988**, *27*, 8097.