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Structure-based design, synthesis, and binding mode analysis of novel and potent chymase inhibitors

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ABSTRACT

Based on insight from the X-ray crystal structure of human chymase in complex with compound **1**, a lactam carbonyl of the diazepane core was exchanged with *O*-substituted oxyimino group, leading to amidoxime derivatives. This modification resulted in highly potent chymase inhibitors, such as *O*-phenylamidoxime **5f**. X-ray crystal structure analysis indicated that compound **5f** induced movement of the Leu99 and Tyr94 side chains at the S2 site, and the increase in inhibitory activity of *O*-phenyl amidoxime derivatives suggested that the *O*-phenyl moiety interacted with the Tyr94 residue. Surface plasmon resonance experiments showed that compound **5f** had slower association and dissociation kinetics and the calculated residence time of compound **5f** to human chymase was extended compared to that of amide compound **1**.

Chymase is a chymotrypsin-like serine protease stored in TC type mast cells that are localized in connective tissue such as the lung, small intestine, and skin.[1,2] Chymase is released from mast cell granules by various stimuli and is involved in inflammatory diseases, including dermatitis,[3,4] allergic conjunctivitis,[5,6] and inflammatory bowel disease.[7] In previous studies of chymase inhibitors as therapeutic agents for atopic dermatitis, we identified 6-benzyl substituted 4-aminocarbonyl-1,4-diazepane-2,5-diones as selective chymase inhibitors.[8,9] Oral dosing of the representative compound SUN13834 improved spontaneous dermatitis in NC/Nga mice.[10] Furthermore, in a mouse dermatitis model induced by repeated painting of 2,4-dinitrofluorobenzene, SUN13834 inhibited not only skin swelling, but also accumulation of inflammatory cells in the skin and significantly decreased scratching behavior.[7] Based on these results, we carried out further structural modifications to improve the inhibitory activity and/or pharmacokinetic profile of this series of compounds and identified novel amidoxime derivatives as potent novel chymase inhibitors. In addition, we found that a substituent on the amidoxime moiety affected the binding mode of the inhibitor to chymase. In this study, we designed and synthesized amidoxime derivatives based on the crystal structure of human chymase in complex with compound **1** (Fig. 1) and analyzed the binding structure and binding kinetics of amidoxime derivatives.

We previously reported the X-ray crystal structure of human chymase in complex with compound **1** (Fig. 1).[9] The key structural features are 1) a benzyl unit located in the hydrophobic S1 pocket, 2) a lactam NH that forms a hydrogen bond with the main chain carbonyl of Ser214, 3) an ethyl moiety orientated in the S1′ site, and 4) amino group and carboxyl group of an anthranic acid, which cooperatively interact with the main chain carbonyl of Phe41 and side chain of Arg143, respectively. Further investigation of the crystal structures of human chymase bound to **1** or related inhibitors revealed the presence of a water molecule in the hydrophobic S2 site comprised of Leu99, Asp102, Ser214, and Tyr215. This water molecule may interact with the carbonyl oxygen at the 2-position of **1** and phenolic hydroxyl group of the Tyr94 side chain. The interaction distances were 3.21 and 2.96 Å, respectively. Examination of other crystal structures of human chymase in complex with our compounds showed that this water molecule was not always observed. In other known X-ray crystal structures of chymase in complex with its inhibitor (e.g. 3S0N, 4K69, 4KP0), this water molecule was also not observed. These findings and the longer distance of these hydrogen bonds suggest that the contribution of the water molecule to binding affinity of **1** is relatively weak. To confirm this prediction, we evaluated the thermodynamic properties of each water molecule[11] in the active site of chymase (details are described in the Supplementary content). Analysis of the

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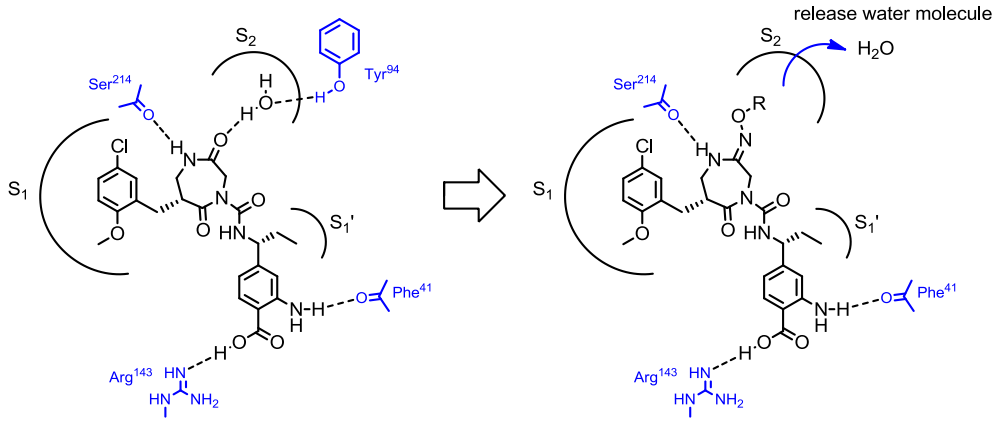


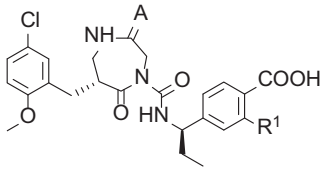
Fig. 1. Schematic view of compound **1** in the active site of human chymase and design of amidoxime derivatives.

calculated interaction energies and excess entropy terms of each hydration site indicated that the water molecule in the hydration site of the S2 region was thermodynamically unfavorable, showing a relatively poor interaction energy despite the large entropy reduction. Based on this result, we expected that displacement of the water molecule with a suitable substituent favorably contributed to the binding free energy, resulting in improved potency. To introduce a substituent that can interact with the S2 site, we designed the *O*-substituted amidoxime derivatives shown in Table 1, maintaining the key hydrogen bond between NH of the seven-membered ring of the inhibitor and of backbone carbonyl Ser214.

The amidoxime compounds in Table 1 were prepared from the known lactams **2a** and **2**c (Scheme 1).[8] To synthesize benzoic acid derivatives **5b** and **5g**, the lactam carbonyl of **2a** was selectively thionated with Belleau's reagent to generate thioamide **3a**, which was converted to amidoxime compounds **4b** and **4g** in a reaction with mercury (II) acetate and the corresponding hydroxylamines. Next, a 2,4,6-trimethoxybenzyl (TMB) group and *tert*-butyl group were deprotected simultaneously under acidic conditions to afford **5b** and **5g**. The benzoic acid derivatives **5c**–**e** were synthesized in the same manner as above using **2b**, which was prepared from **2a** by deprotection of the TMB group with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ). This method, including initial deprotection of the TMB group, has the advantage that final product is easily isolated and purified, although this results in an additional synthetic step. In the case of anthranic acid compounds **5a** and **5f**, the TMB group of compound **2c** was initially deprotected by DDQ, and then the nitro group was reduced with zinc powder in acetic acid to give compound **2d**, which was converted to compounds **5a** and **5f** in the same manner as above. The reference compound **6** was prepared from **2a** by deprotection of the TMB group and *tert*-butylester under acidic condition.

The compounds shown in Table 1 were evaluated for their inhibitory activity against recombinant human chymase (detailed assay conditions are described in the Supplementary content). *O*-Ethylamidoximes **5a** and **5b** showed more potent inhibitory activities than the corresponding amide compounds **1** and **6**, as expected. In the both cases of the amide (**1** and **6**) and amidoxime (**5a** and **5b**) derivatives, introduction of amino group to benzoic acid moiety slightly increase the inhibitory activity. Although the hydrogen bond between the amino group and Phe41 main chain would contribute to improve the potency, we initially examined the substituent effect of amidoxime moiety by using readily prepared benzoic acid derivatives (R^1 = H). As a result, the inhibitory activity of bulkier *O*-alkyl amidoximes such as isopropyl **5c**, isobutyl **5d**, and cyclopentyl **5e** decreased as the size of the alkyl

Table 1
Inhibitory activity of amidoxime derivatives against human chymase.[a]



Compound	R^1	A	IC_{50} (μM)
1	NH_2	O	0.30
6	H	O	0.57
5a	NH_2	N-O-Et	0.078
5b	H	N-O-Et	0.24
5c	H	N-O-*iso*Pr	0.58
5d	H	N-O-*iso*Bu	1.2
5e	H	N-O-cyclopentyl	2.4
5f	NH_2	N-O-Ph	0.0089
5g	H	N-O-Ph	0.050

[a] Detailed assay conditions are described in the Supplementary content.

substituent increased. However, in contrast to the alkyl substituents, the bulky *O*-phenylamidoxime **5g** showed more potent inhibitory activities. Furthermore, **5f**, the anthranic acid derivative of *O*-phenylamidoxime, exhibited the most potent, a one digit nanomolar activity. This result shows the importance of amino group of anthranic acid moiety again, and also the preservation of hydrogen bond with Phe41 in amidoxime derivatives.

To understand these substituent effects, we examined the X-ray crystal structures of *O*-ethylamidoxime **5b** and *O*-phenylamidoxime **5f** bound to human chymase (Figs. 2 and 3). The X-ray crystal structure of *O*-ethylamidoxime **5b** in the active site of human chymase (Fig. 2a, PDB ID: 5YJP) revealed that nearly all key interactions were maintained compared to that of amide compound **1**: 1) the benzyl unit occupies the S1 pocket, 2) the lactam NH forms a hydrogen bond with Ser214, 3) the ethyl moiety is located in the S1′ site, and 4) the benzoic acid interacts with Arg143. At the S2 site, the Leu99 side chain slightly moves and accepts the ethyl moiety of compound **5b** (Fig. 3a). Therefore, the water molecule is released into bulk solution, as expected. The increase in inhibitory activity of **5b** may be attributable to the free energy gain from the release of water molecules. Since the inhibitory activity decreased as the size of alkyl substituent of amidoxime moiety was increased, it is speculated that the movement of the Leu99 side chain is restricted. In the case of the complex structure with *O*-phenylamidoxime **5f** (Fig. 2b, PDB ID: 5YJM), it is observed that in addition to the movement of Leu99,