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Identification of a Stable Chymase Inhibitor Using a Pharmacophore-Based Database Search

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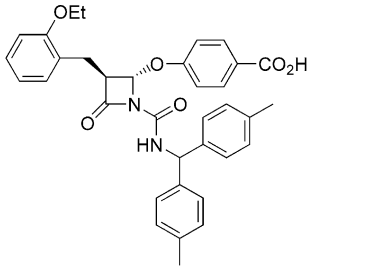
Abstract—In general, serine protease chymase inhibitors readily decompose in plasma. We previously found that thiazolidine-2,4-dione and thiadiazole derivatives are also unstable. Using a pharmacophore-based database search, we identified a benzo[*b*]thiophen-2-sulfonamide derivative as a stable chymase inhibitor. Finding a lead compound with adequate activity and stability by a pharmacophore-based approach is more efficient than modifying an unstable compound to reduce its instability without simultaneously decreasing its inhibitory activity. Our pharmacophore model of chymase inhibitors suggests that the two hydrophobic interactions in the S1 and S1′ regions and the two H-bonding interactions between them play important roles in chymase inhibitors.

Chymase (EC 3.4.21.39) is a major chymotrypsin-like serine protease that is expressed in the secretory granules of mast cells in many mammalian species.[1–4] Chymase can convert angiotensin (Ang) I to Ang II more efficiently than Ang I-converting enzyme (ACE).[5] The localized production of Ang II by human chymase in the cardiovascular system has been shown to influence cardiovascular disease.[6–11] IgE and mast cell chymase have been shown to play a key role in atopic or allergic inflammation and fibroproliferation in the skin.[12,13]

Due to their lack of stability, almost all previously reported chymase inhibitors have shown no potency in vivo. Recently, orally active chymase inhibitors have been investigated, and they have been shown to be potent therapeutic agents (Fig. 1).[14–23] While even these stable candidates have problems regarding stability, this can be improved by structural modification. It would be difficult to recognize which features of peptidic-mimetics or compounds identified by random screening, such as BCEAB, make them suitable as chymase inhibitors and a great deal of effort would be needed to determine which are stable.



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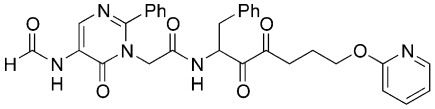

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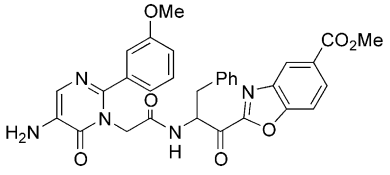

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Figure 1. Reported orally active chymase inhibitors.

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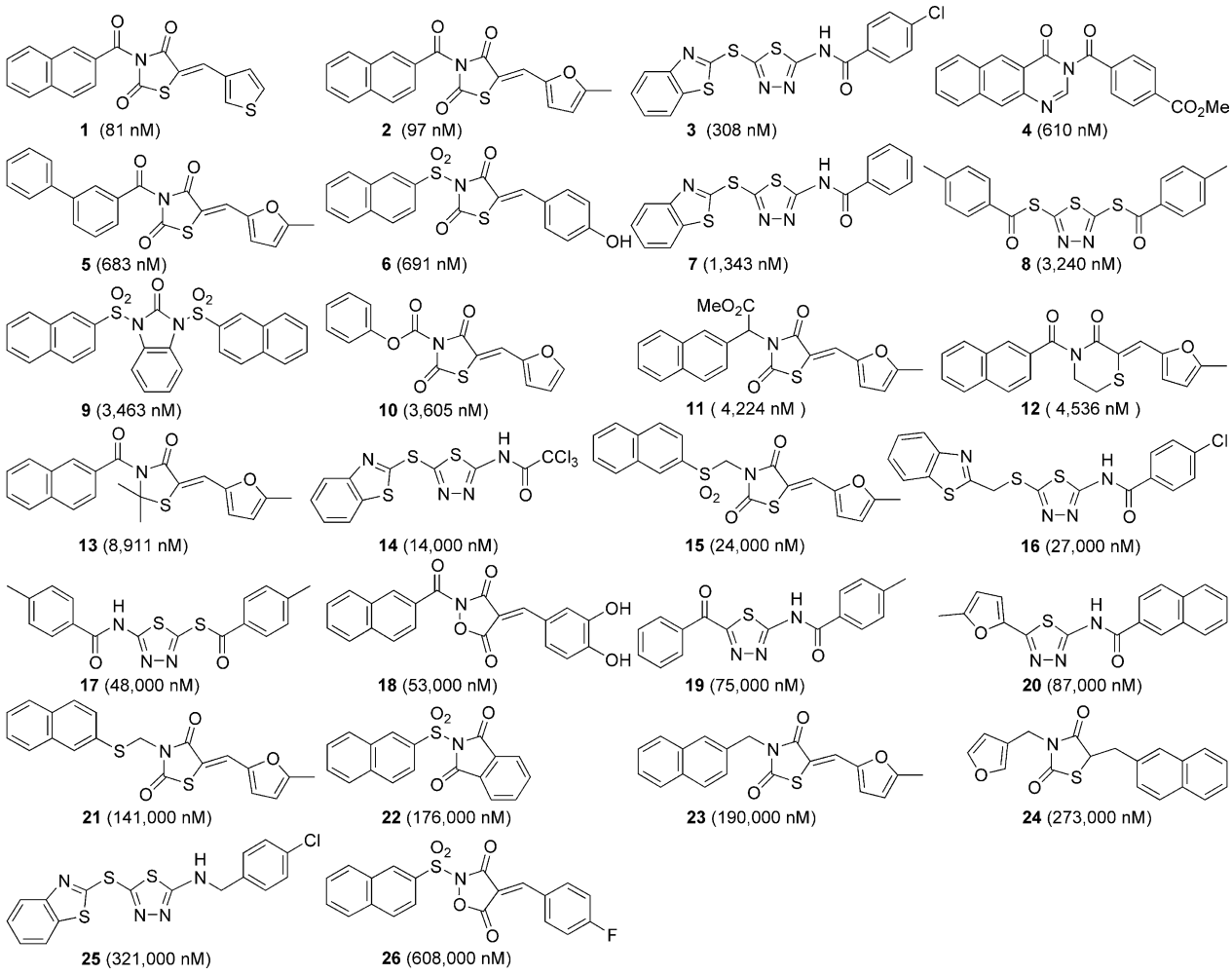

Figure 2. Training set consisting of our chymase inhibitors.

We found that thiazolidine and the thiadiazole derivatives shown in Figure 2 were useful chymase inhibitors, but they were also unstable and completely decomposed in human plasma upon incubation for 60 min at 37 °C. Although we studied further structural modifications, we could not improve their stability without decreasing their inhibitory activity.

To efficiently identify a stable chymase inhibitor that is different from our unstable compounds, we generated a pharmacophore model that described the structural requirements of a chymase inhibitor and searched a database using Catalyst® software.[24] The pharmacophore model of a chymase inhibitor was generated using our compounds and their assay results. The training set, consisting of 26 compounds, gave the best results with respect to a cost analysis in Catalyst: null-fixed cost = 54.07 and null-1st hypothesis cost = 36.70 (Fig. 3). The 1st hypothesis model showed a strong correlation with a training set consisting of the 26 compounds in Figure 2 ($r = 0.8781$). This model also showed a good correlation with a test set consisting of 60 compounds that were not used for hypothesis generation ($r = 0.7542$). Since these correlations were the best among the 10 hypothesis models examined, we used the 1st hypothesis model for the rest of the study. This model consisted of two hydrophobes at both ends and two H-bond acceptors (HBA) between them. The ACD database (available Chemical Directory, MDL Information Systems, Inc.; 216,599 compounds) was searched using this model, and we selected 45 of the retrieved compounds for screening. Of these, three compounds showed inhibitory activity of more than 30% at 1 μM (Fig. 4). While Maybridge KM 01221 and

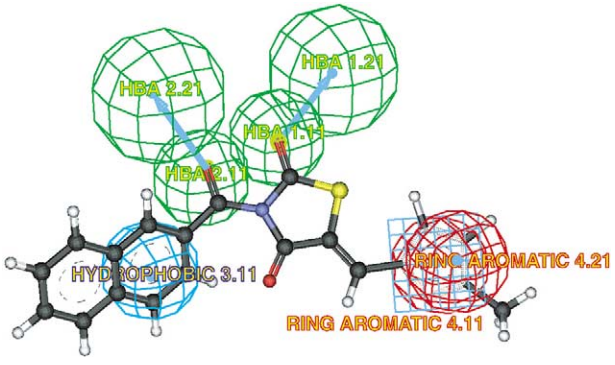

Figure 3. Hypothesis model of a chymase inhibitor superimposed on compound **2** (HBA = H-bond acceptor). The estimated activity was 34 nM and the conformational energy relative to the minimum-energy conformer (ΔE) was 2.63 kcal/mol.

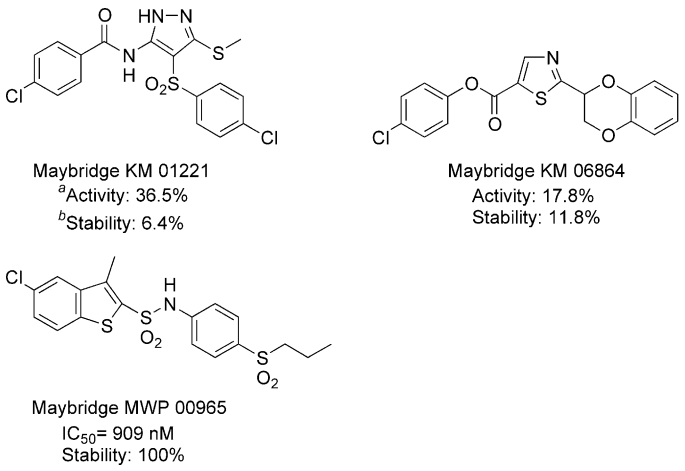

Figure 4. Active compounds that showed more than 30% inhibition at 1 μM. [a]Inhibitory activity was defined as percent inhibition by the test compound at 1 μM. [b]Stability was defined as percent remaining in human plasma after incubation for 60 min at 37 °C.

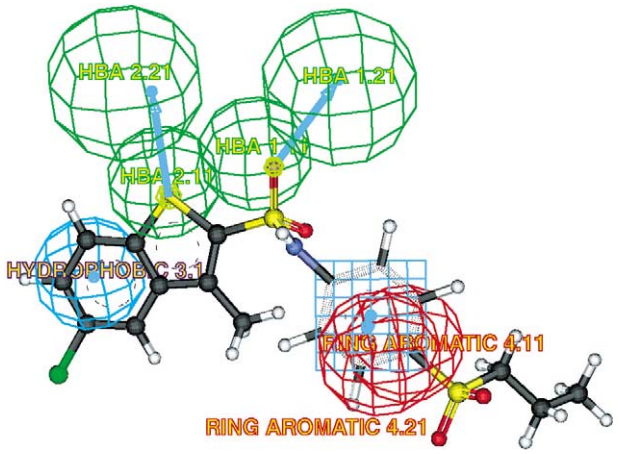

Figure 5. Hypothesis model of a chymase inhibitor superimposed on MWP 00965. Estimated activity was 6200 nM and ΔE was 8.41 kcal/mol.

KM 06864 were unstable, like thiazolidine derivatives, Maybridge MWP 00965, a benzo[*b*]thiophen-2-sulfonamide derivative, showed no significant decrease over 60 min and was as potent a chymase inhibitor as the lead compound. This compound is suitable as a starting lead because of its simplicity and stability. MWP 00965 was fit into the hypothesis model, and this suggested the need for thiophen as an HBA (Fig. 5).

We also considered docking models of compound 2 and MWP 00965 with chymase independent of the hypothesis model (Fig. 6). Docking models suggested that hydrophobic 3.11 and the aromatic ring 4.11 in the hypothesis model would interact with the S1 and S1′ regions in chymase.[25] The aryl groups in the S1 pockets of compound 2 and MWP 00965 were similar. The sulfonamide of MWP 00965 would stabilize the interaction through two H-bonds more efficiently than that of compound **2**. The important H-bond interaction with Ser195 could be described as HBA2.11 in compound **2**. The role of thiophen in MWP 00965, which was evaluated as HBA2.11, was unclear. We speculated that it could form an H-bond with Ser195. Otherwise, the propylsulfonyl substituent of MWP 00965 would not fit in S1′. Therefore, we identified a possible means to improve the inhibitory activity of MWP 00965 by modifying the substituent at the *p*-position of the phenyl group.

Our hypothesis model showed an important interaction with chymase, and that MWP 00965 was stable and useful as a chymase inhibitor. Considering its binding model, we found that the benzo[*b*]thiophen-2-sulfonamide moiety was useful in the S1 region of chymase. Further modification of this benzo[*b*]thiophen-2-sulfonamide derivative is now underway.

Computational Methods

Catalyst 4.5

Conformational models were calculated using an energy cutoff of 15 kcal for the best quality. The number of conformers generated for each molecule was limited to a maximum of 255. H-bond acceptor (HBA), hydrophobic and aromatic ring features were used for HypoGen hypothesis generation. The spacing parameter was 200 picometers. The database search was conducted using a fast flexible search, and compounds with a fit value of less than 10% and a molecular weight of more than 500 were omitted from the hit list.

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The crystal structure of chymase with PMSF (PDB code 1KLT)[23] was used as a template. All water molecules were removed for calculation. The chymase backbone was fixed during calculation. A distance constraint between C of the carbonyl on the naphthoyl ring in compound **2** or S of the sulfonamide in MWP 00965 and oxygen of Ser195 was applied using a flat-bottom potential with a force constant of 0.0 kcal/mol for a distance of 0–2.5 Å and 100.0 kcal/mol for longer

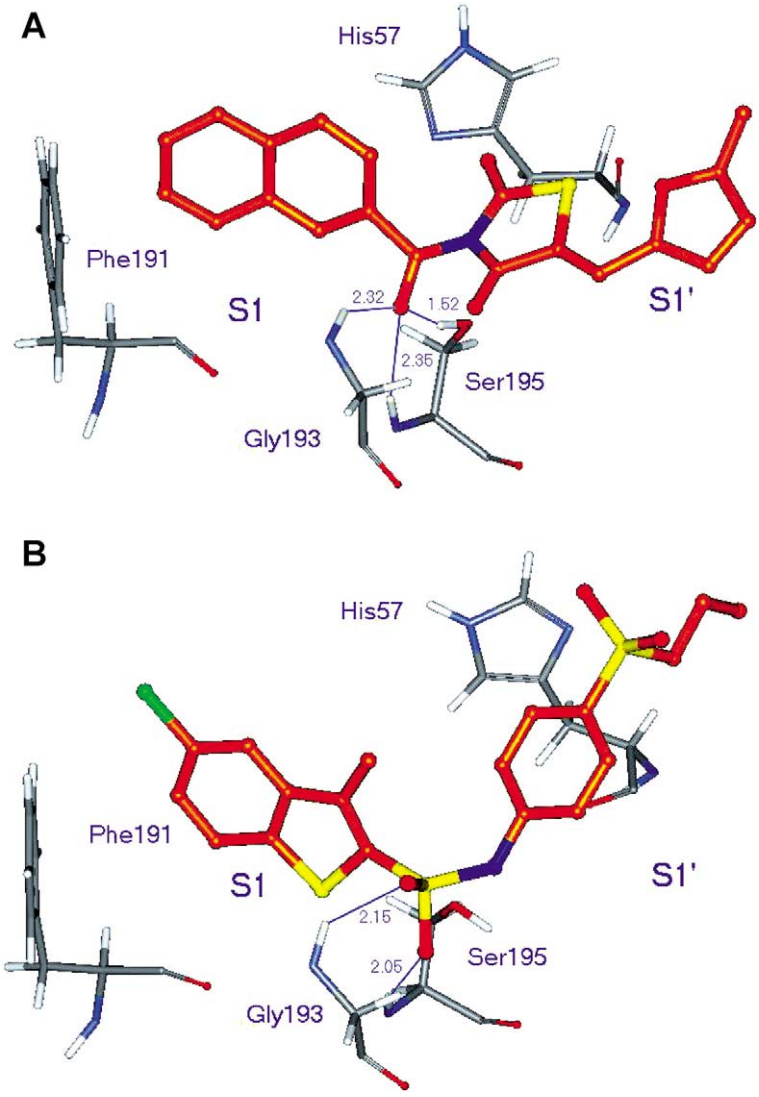

Figure 6. Docking model of inhibitors with chymase (A) docking model of compound **2**; (B) docking model of MWP 00965.

distances. One hundred molecular dynamics cycles, consisting of 50 ps at 900 K in an NVT ensemble, were performed using a cvff force field. After minimization by a combination of steepest-descent and conjugate gradient minimization algorithms until the maximum rms derivative was less than 0.01 kcal/Å, the lowest-energy conformation was chosen.

Acknowledgements

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References and Notes

1. Takai, S.; Shiota, N.; Sakaguchi, M.; Muraguchi, H.; Matsumura, E.; Miyazaki, M. *Clin. Chim. Acta* **1997**, *264*, 13.
2. Caughey, G. H.; Raymond, W. W.; Wolters, P. J. *Biochim. Biophys. Acta* **2000**, *1480*, 245.
3. Takai, S.; Shiota, N.; Yamamoto, D.; Okunishi, H.; Miyazaki, M. *Life Sci.* **1996**, *58*, 591.
4. Wintroub, B. U.; Schechter, N. B.; Lazarus, G. S.; Kaempfer, C. E.; Schwartz, L. B. *J. Invest. Dermatol.* **1984**, *83*, 336.
5. Ihara, M.; Urata, H.; Kinoshita, A.; Suzumiya, J.; Sasaguri, M.; Kikuchi, M.; Ideishi, M.; Arakawa, K. *Hypertension* **1999**, *33*, 1399.
6. Nishimoto, M.; Takai, S.; Kim, S.; Jin, D.; Yuda, A.; Sakaguchi, M.; Yamada, M.; Sawada, Y.; Kondo, K.; Asada, K.; Iwao, H.; Sasaki, S.; Miyazaki, M. *Circulation* **2001**, *104*, 1274.
7. Takai, S.; Yuda, A.; Jin, D.; Nishimoto, M.; Sakagichi, M.; Sasaki, S.; Miyazaki, M. *FEBS Lett.* **2000**, *467*, 141.
8. Takai, S.; Shiota, N.; Kobayashi, S.; Matsumura, E.; Miyazaki, M. *FEBS Lett.* **1997**, *412*, 86.
9. Shiota, N.; Fukamizu, A.; Takai, S.; Okunishi, H.; Murakami, K.; Miyazaki, M. *J. Hypertens.* **1997**, *15*, 431.
10. Shiota, N.; Jin, D.; Takai, S.; Kawamura, T.; Koyama, M.; Nakamura, N.; Miyazaki, M. *FEBS Lett.* **1997**, *406*, 301.
11. Urata, H.; Kinoshita, A.; Misono, K. S.; Bumpus, F. M.; Husain, A. *J. Biol. Chem.* **1990**, *265*, 22348.
12. Pascale, E.; Tarani, L.; Meglio, P.; Businco, L.; Battiloro, E.; Cimino-Reale, G.; Verna, R.; D'Ambrosio, E. *Hum. Hered.* **2001**, *51*, 177.

13. Kakizoe, E.; Shiota, N.; Tanabe, Y.; Shimoura, K.; Kobayashi, Y.; Okunishi, H. *J. Invest. Dermatol.* **2001**, *116*, 118.
14. Akahoshi, F.; Ashimori, A.; Yoshimura, T.; Imada, T.; Nakajima, M.; Mitsutomi, N.; Kuwahara, S.; Ohtsuka, T.; Fukaya, C.; Miyazaki, M.; Nakamura, N. *Bioorg. Med. Chem.* **2001**, *9*, 301.
15. Akahoshi, F.; Ashimori, A.; Sakashita, H.; Yoshimura, T.; Imada, T.; Nakajima, M.; Mitsutomi, N.; Kuwahara, S.; Ohtsuka, T.; Fukaya, C.; Miyazaki, M.; Nakamura, N. *J. Med. Chem.* **2001**, *44*, 1286.
16. Akahoshi, F.; Ashimori, A.; Sakashita, H.; Yoshimura, T.; Eda, M.; Imada, T.; Nakajima, M.; Mitsutomi, N.; Kuwahara, S.; Ohtsuka, T.; Fukaya, C.; Miyazaki, M.; Nakamura, N. *J. Med. Chem.* **2001**, *44*, 1297.
17. Aoyama, Y.; Uenaka, M.; Kii, M.; Tanaka, M.; Konoike, T.; Hayasaki-Kajiwara, Y.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem.* **2001**, *9*, 3065.
18. Aoyama, Y.; Uenaka, M.; Konoike, T.; Iso, Y.; Nishitani, Y.; Kanda, A.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2397.
19. Aoyama, Y.; Uenaka, M.; Konoike, T.; Iso, Y.; Nishitani, Y.; Kanda, A.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2403.
20. Fukami, H.; Imajo, S.; Ito, A.; Kakutani, S.; Shibata, H.; Sumida, M.; Tanaka, T.; Niwata, S.; Saitoh, M.; Kiso, Y.; Miyazaki, M.; Okunishi, H.; Urata, H.; Arakawa, K. *Drug Des. Discov.* **2000**, *17*, 69.
21. Jin, D.; Takai, S.; Yamada, M.; Sakaguchi, M.; Miyazaki, M. *Life Sci.* **2002**, *71*, 437.
22. Takai, S.; Jin, D.; Nishimoto, M.; Yuda, A.; Sakaguchi, M.; Kamoshita, K.; Ishida, K.; Sukenaga, Y.; Sasaki, S.; Miyazaki, M. *Life Sci.* **2001**, *69*, 1725.
23. Takai, S.; Jin, D.; Sakaguchi, M.; Kirimura, K.; Miyazaki, M. *Jpn. J. Pharmacol.* **2001**, *86*, 124.
24. *Rational Drug Design Software Catalyst*®; Accelrys: San Diego, CA, USA. See: http://www.accelrys.com/catalyst/index.html and http://www.accelrys.com/references/rdd_pub.html#catalyst.
25. McGrath, M. E.; Mirzadegan, T.; Schmidt, B. F. *Biochemistry* **1997**, *36*, 14318.