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Total Synthesis of Human Chymase Inhibitor Methyllinderone and Structure–Activity Relationships of Its Derivatives

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Abstract—Total synthesis of human chymase inhibitor methyllinderone has been achieved in only four steps with an overall yield of 21% from dimethyl squarate. We developed an efficient synthetic method for obtaining methyllinderone derivatives and found the active compound. In addition, we propose the inhibition mechanism of the active compound against human chymase using calculations.

Introduction

Human chymase is a chymotrypsin-like serine protease that is stored in the secretory granules of mast cells.[1] Although the precise physiological and pathological functions of human chymase have not been elucidated, several studies have suggested its involvement in cardiovascular diseases[2] and chronic inflammation following fibrosis.[3] Chymase inhibitors[4] are thought to be potentially useful as tools for elucidating the physiological function of chymase and therapeutic agents.

Screening of our compound library led to identification of methyllinderone **1** isolated from *Lindera erythrocarpa* Makino (Lauraceae)[5] as a human chymase inhibitor (IC_{50} 30 μM). Methyllinderone belongs to a unique class of natural acylcyclopentendione pigments.[5,6] To the best of our knowledge, this is the first report of the biological activity of methyllinderone. We describe herein an efficient total synthesis of methyllinderone **1**, structure–activity relationship studies of its derivatives, and the inhibition mechanism of active compound **12** against human chymase.

Chemistry

The total synthesis of methyllinderone **1** was achieved by the Lee group via biogenetic ring-contraction reaction.[7] However, their total synthesis consists of many steps. We therefore tried to develop a short-step total synthesis with the key step being a palladium-catalyzed ring-expansion reaction (Scheme 1).

Cyclobutenone **4** was obtained by treatment of dimethyl squarate **2** with lithium acetylide of alkyne **3** prepared from hydrocinnamaldehyde by the Corey–Fuchs method.[8] The ring expansion reaction of **4** with 5% $Pd(OCOCF_3)_2$ in the presence of *N*-bromosuccinimide (NBS) provided compound **5** in 77% yield.[9] Dehydrogenation of **5** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[7] followed by nucleophilic substitution with NaOMe gave methyllinderone **1**.[10] Total synthesis of methyllinderone **1** could be achieved in only four steps, which was much shorter than Lee's method,[7] and the overall yield from dimethyl squarate **2** was 21%.

We next investigated the short-step synthesis of methyllinderone derivatives to study structure–activity relationships in the inhibition of human chymase (Scheme 2).

Compound **9**, in which three methoxy groups were removed from methyllinderone **1**, was obtained by treatment with 4-cyclopenten-1,3-dione **7** and cinnamaldehyde **8** in the presence of excess (20 equiv) $BF_3{\cdot}OEt_2$ in only one step (52% yield). The presence of bases (1,8-diazabicyclo[5.4.0]undec-7-ene, Et_3N or NaOMe) or *p*-toluenesulfonic acid[11] provided a complex mixture or a low yield, respectively. Monomethoxy compound **12** was prepared as follows. Compound **11**

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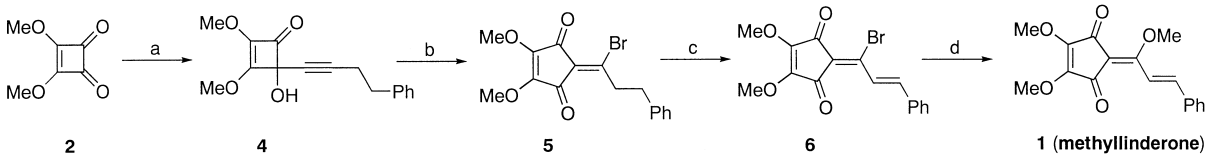
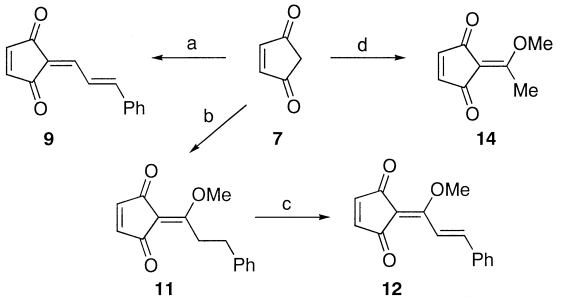


Scheme 1. Reagents and conditions: (a) $PhCH_2CH_2CCH$ (**3**), *n*-BuLi, THF (70%); (b) $Pd(OCOCF_3)_2$, NBS, CH_2Cl_2; (c) DDQ, PhCl (53%); (d) NaOMe, MeOH (72%).



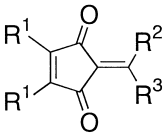
Scheme 2. Reagents and conditions: (a) PhCH=CHCHO (8), $BF_3{\cdot}OEt_2$, reflux (52%); (b) $PHCH_2CH_2C(OMe)_3$ (**10**), $ZnCl_2$, Ac_2O (30%); (c) DDQ, PhCl (41%); (d) $MeC(OMe)_3$ (**13**), $ZnCl_2$, Ac_2O (46%).

was obtained by treatment of **7** with orthoester **10**, which was prepared from hydrocinnamonitrile by a known procedure,[12] in the presence of $ZnCl_2$.[13] Dehydrogenation of **11** with DDQ provided compound **12** (two steps, 12%). Compound **14** was gained from **7** and orthoester **13** by the above procedure (46%).[13,14]

Results and Discussion

The human chymase inhibition data are presented in Table 1.[15] Compound **12** was the most active compound (IC_{50} 1.7 μM) and 18-fold more potent than methyllinderone **1**. Removal of the R^2-methoxy group afforded a 7-fold reduction in activity (**9** vs **12**). Conversion of cinnamyl group **12** to hydrocinnamyl group **11** led to 24-fold decrease of potency. Compound **14** showed no potency.

Table 1. Chymase inhibitory activity



Compd	R^1	R^2	R^3	IC_{50} (μM)
1	OMe	OMe	CH=CHPh	30
9	H	H	CH=CHPh	12
11	H	OMe	CH_2CH_2Ph	40
12	H	OMe	CH=CHPh	1.7
14	H	OMe	Me	> 1000

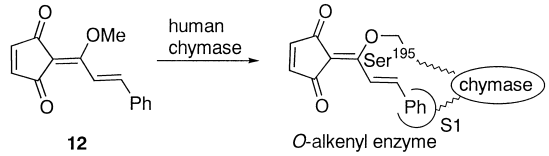


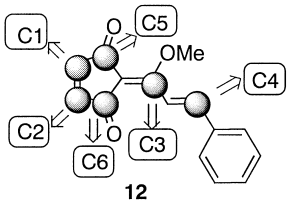
Figure 1. Proposed mechanism for inhibition of human chymase by compound **12**.

Judging from the results shown in Table 1 and the docking study on the binding of 1-oxacephem to human chymase,[4a] we assumed that the inhibition mechanism of compound **12** against human chymase was as presented in Figure 1, which shows that nucleophilic substitution of the active site serine residue (serine 195) in the enzyme at the β-olefinic carbon atom activated by two carbonyl groups leads to the generation of serine hydroxy *O*-alkenyl enzyme while the phenyl group docks into the S1 pocket.

The following experiments were performed in order to support the proposed mechanism presented in Figure 1: (1) calculations of the LUMO coefficients of compound **12** (Table 2);[16] (2) overlap of compound **12** with hemiketal intermediate **16** derived from 1-oxacephem human chymase inhibitor **15** reported in the previous paper (Figs. 2 and 3).[4a,17]

Table 2 suggests that the C3-carbon is electronically the most reactive against nucleophilic attack of the active serine hydroxyl group. Figure 2 shows that the C8-carbon of **16**[17] (the β-lactam carbonyl carbon of **15**) and the C3-carbon of **12** overlap each other when the phenyl ring of **12** is superimposed on the 7β-phenyl ring of **16**, namely, the C3-carbon is situated near the active serine hydroxy group when the phenyl group of compound **12** is enclosed by the residue of the S1 pocket. The above results (Table 2 and Fig. 3) offer support for the hypothesis presented in Figure 1. Accordingly, the decrease of potency of compounds **9** and **11** compared with compound **12** is considered to be ascribed to the decrease of electrophilicity of the C3-carbon of **9** and **11** because of the lack of R^2-methoxy group or dienone structure. The lack of hydrophobic interaction between the R^3-methyl group and the residue of the S1 pocket leads to no potency of compound **14**.

Table 2. Calculations of the LUMO coefficients of compound **12**



LUMO coefficients		LUMO coefficients	
C1	−0.077	C4	+0.438
C2	−0.066	C5	+0.173
C3	−0.495	C6	+0.186

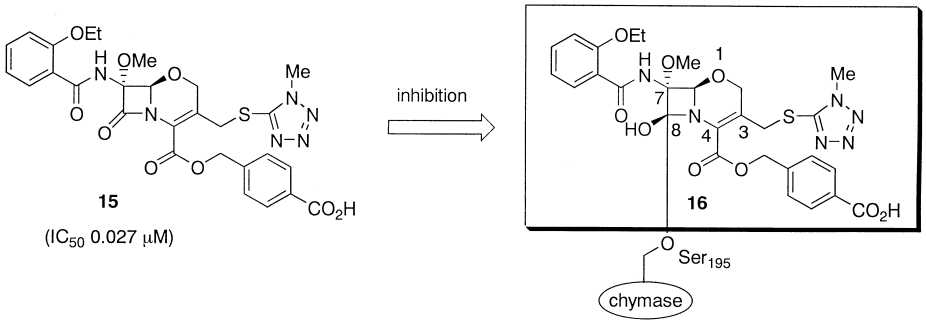


Figure 2. 1-Oxacephem human chymase inhibitor **15** and hemiketal intermediate **16**.

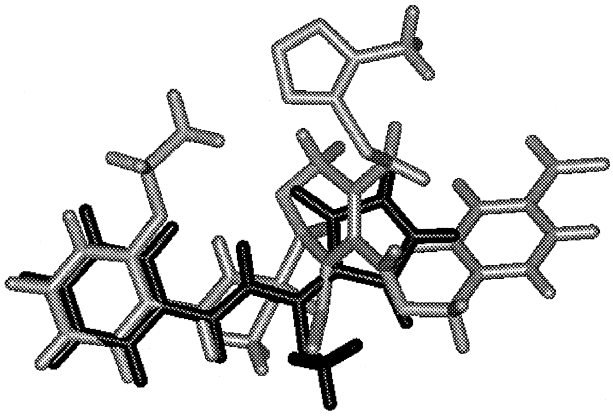


Figure 3. Overlap of compound **12** (black) with hemiketal intermediate **16** (gray).

Conclusion

We have achieved a short-step total synthesis of chymase inhibitor methyllinderon **1**. We also developed an efficient synthetic method for methyllinderon derivatives, and identified compound **12** as an active chymase inhibitor. Finally, we have proposed the inhibition mechanism of compound **12** against human chymase.

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References and Notes

1. Wang, Z.; Walter, M.; Selwood, T.; Rubin, H.; Schechter, N. M. *Biol. Chem.* **1998**, *379*, 167.
2. Ihara, M.; Urata, H.; Kinoshita, A.; Suzumiya, J.; Sasaguri, M.; Kikuchi, M.; Ideishi, M.; Arakawa, K. *Hypertension* **1999**, *33*, 1399.
3. (a) Hamada, H.; Terai, M.; Kimura, H.; Hirano, K.; Oana, S.; Niimi, H. *Am. J. Respir. Crit. Care. Med.* **1999**, *160*, 1303. (b) Takai, S.; Yuda, A.; Jin, D.; Nishimoto, M.; Sakaguchi, M.; Sasaki, S.; Miyazaki, M. *FEBS Lett.* **2000**, *467*, 141.
4. (a) Aoyama, Y.; Uenaka, M.; Konoike, T.; Iso, Y.; Nishitani, Y.; Kanda, A.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2397. Aoyama, Y.; Uenaka, M.; Konoike, T.; Iso, Y.; Nishitani, Y.; Kanda, A.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2403. (b) 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. (c) Hayashi, Y.; Iijima, K.; Katada, J.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 199. (d) Groutas, W. C.; Schechter, N. M.; He, S.; Yu, H.; Huang, P.; Tu, J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2199. (e) Iijima, K.; Katada, J.; Hayashi, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 413. (f) Fukami, H.; Okunishi, H.; Miyazaki, M. *Curr. Pharm. Des.* **1998**, *4*, 439 and references therein.
5. The IR, MS, and ^{1}H NMR data of methyllinderone **1** isolated from *Lindera erythrocarpa* Makino (Lauraceae) were identical with those reported by Leong (Leong, Y.-W.; Harrison, L. J.; Bennett, G. J.; Kadir, A. A.; Connolly, J. D. *Phytochemistry* **1998**, 47, 891).
6. Kiang, A. K.; Lee, H. H.; Sim, K. Y. *J. Chem. Soc.* **1962**, 4338.
7. (a) Lee, H. H.; Tan, C. H. *J. Chem. Soc., Sect. C* **1967**, 1583. (b) Bennett, G. J.; Lee, H. H. *J. Chem. Soc., Perkin Trans. 1* **1986**, 633.
8. Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, *13*, 3769.
9. Liebeskind, L. S.; Mitchell, D.; Foster, B. S. *J. Am. Chem. Soc.* **1987**, *109*, 7908.
10. The IR, MS, and ^{1}H NMR data of synthetic methyllinderone **1** were identical with those reported by Leong.[5]
11. Inayama, S.; Mamoto, K.; Tetsuichi, S.; Hirose, T. *J. Med. Chem.* **1976**, *19*, 433.
12. Casy, G.; Patterson, J. W.; Taylor, R. J. K. *Org. Synth.* **1988**, *67*, 193.
13. Miki, T.; Hiraga, K.; Asako, T.; Masuya, H. *Chem. Pharm. Bull.* **1967**, *15*, 670.
14. The structures of methyllinderone derivatives **9**, **11**, **12**, and **14** were confirmed by ^{1}H NMR, IR, and mass spectrometric analysis.
15. The human chymase assay was performed as follows. First, human chymase was purified according to the method of Takai (Takai, S.; Siota, N.; Sakaguchi, M.; Muraguchi, H.; Matsumura, E.; Miyazaki, M. *Clin. Chim. Acta* **1997**, *265*, 13). The purified chymase was preincubated with test compounds dissolved in DMSO at 37 °C for 30 min in 0.1 M Tris–HCl (pH 8.0) containing 1.8 M NaCl, after then the chymase reaction was started by adding succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilides (Sigma Chemical Co.). The change of absorbance was measured at 405 nm after 2 h incubation at 37 °C. The IC_{50} value was calculated from the inhibition of *p*-nitroaniline formation at each concentration of the test compound.
16. The three-dimensional model of compound **12** was constructed based on the published X-ray structure of sodium lucidonate (Takai, M.; Liu, S.-Y.; Ogihara, Y.; Iitaka, Y. *Chem. Pharm. Bull.* **1977**, 25, 1404). Low energy conformation, the LUMO coefficients of compound **12** were calculated using the AM1 semiempirical method as implemented in the MOPAC version 6.0 system.
17. See ref 4a for the three-dimensional model of hemiketal intermediate **16** derived from 1-oxacephem **15**.