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Inhibition of Serine Proteases: Activity of 1,3-Diazetidine-2,4-diones

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Abstract—The present work demonstrates that the 1,3-diazetidine-2,4-dione nucleus is effective as a scaffold of serine protease inhibitors. Compound **1** displayed high activity against human cathepsin G and α-chymotrypsin (0.39, 0.69 nM). Compound **6** exhibited 0.85 nM inhibition of human chymase. Compound **10** was a selective inhibitor against human neutrophil elastase. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Serine proteases take part in various physiological as well as pathological processes and are hence important and interesting therapeutic targets. We have investigated a new class of inhibitors of human chymase,[1] one of the chymotrypsin-like serine proteases, which is involved in the generation of angiotensin II from angiotensin I[2] and activation of precursor interleukin-1β.[3]

Screening of our compound collection led to identification of 1,3-diazetidine-2,4-dione **1** as a chymase inhibitor (IC_{50} 4.0 nM). We then examined biological activities of 1,3-diazetidine-2,4-dione derivatives against other serine proteases and found 1,3-diazetidine-2,4-dione derivatives to possess high activities against bovine pancreatic α-chymotrypsin, human cathepsin G and human neutrophil elastase. However, little has been reported about the biological activities of 1,3-diazetidine-2,4-dione derivatives. We describe herein the effectiveness of the 1,3-diazetidine-2,4-dione nucleus as a scaffold for inhibition of serine proteases including human chymase.

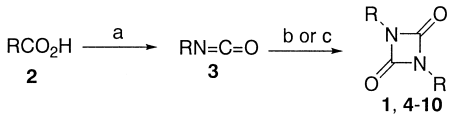
Chemistry

1,3-Diazetidine-2,4-dione derivatives were prepared as follows (Scheme 1). Isocyanates **3** were prepared from the corresponding carboxylic acids **2** by the Shioiri method.[4] Compounds **1** and **4**–**9** were obtained by dimerization of isocyanates **3** in the presence of 1,2-dimethylimidazole.[5] Dimerization of ethyl isocyanate gave compound **10** in the presence of $SbCl_5$ (Table 1).[6,7]

Results and Discussion

First, we investigated the inhibitory activities of 1,3-diazetidine-2,4-dione derivatives against human chymase (Table 1).[8] Compound **6** exhibited excellent activity (IC_{50} 0.85 nM). Generally, *N*-benzyl derivatives showed high activity against human chymase (**1** and **4**–**6**). However, *N*-aryl derivatives **7** and **8** are less potent than *N*-benzyl derivatives, and *N*-alkyl derivatives **9** and **10** are not active against human chymase.

Next, we examined inhibitory activities of 1,3-diazetidine-2,4-dione derivatives against seven kinds of serine proteases. The results are summarized in Table 2 together with the above data with respect to human chymase.[9] Compounds **1** and **7** showed higher activities



Scheme 1. Reagents and conditions: (a) Et_3N, $(PhO)_2P(O)N_3$; (b) 1,2-dimethylimidazole, CH_2Cl_2; (c) $SbCl_5$, CH_2Cl_2.

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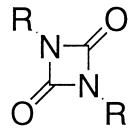
against bovine pancreatic α-chymotrypsin and human cathepsin G. It is noteworthy that compound **10** was a selective inhibitor against human neutrophil elastase.

The above results (Tables 1 and 2) suggest the following with regard to the inhibition mechanism of 1,3-diazetidine-2,4-dione derivatives against serine proteases (Fig. 1):[10] (1) 1,3-diazetidine-2,4-dione nucleus docks at the active site of these serine proteases and one of the *N*-substituents of 1,3-diazetidine-2,4-dione is located at the prime specificity site S_1 of a target enzyme and determines the selectivity against serine enzymes; (2) the active site serine hydroxy group in the enzyme launches a nucleophilic attack against one of carbonyl groups of the 1,3-diazetidine-2,4-dione nucleus and consequently an acylated enzyme is generated.

The primary substrate specificity of human chymase is Phe and Tyr.[11] This agrees with the results that *N*-benzyl derivatives **1** and **4**–**6** display high activities against human chymase (Table 1). The primary substrate specificities of human cathepsin G (Phe)[12] and human elastase (Val, Leu)[13] contribute to the improvement in the potency of compound **1** against cathepsin G and the selectivity of compound **10** against elastase (Table 2).

Table 1. Potency of 1,3-diazetidine-2,4-dione derivatives against human chymase



Compd	R	Isolated yields from **3** (%)	IC_{50} (nM)
1	$C_6H_5CH_2$	70	4.0
4	4-MeO-$C_6H_4CH_2$	42[a]	2.0
5	3-MeO-$C_6H_4CH_2$	20[a]	2.3
6	3,4-OCH_2O-$C_6H_3CH_2$	20[a]	0.85
7	4-Me-C_6H_4	90	140
8	3,4-OCH_2O-C_6H_3	42[a]	48
9	EtO_2CCH_2	62	na[b]
10	Et	18	na

[a]Isolated yields from **2**.
[b]No activity. $IC_{50} > 10{,}000$ nM.

Finally, we tried to determine why the 1,3-diazetidine-2,4-dione nucleus is effective as an inhibitor scaffold[14] for serine proteases. A typical β-lactam compound **11** was prepared as an analogue of **1** (Table 3).[15] In spite of structural similarity, compounds **11** showed no inhibition against all serine proteases presented in Table 2 ($IC_{50} > 10{,}000$ nM). Further, compared with the chemical stability of each compound in sodium methoxide, 10 mM solution in methanol, compound **11** was much more stable than compound **1**.[16] These results suggest that 1,3-diazetidine-2,4-dione nucleus is much more reactive than azetidine-2-one. As presented in Figure 2, large differences of potency between compounds **1** and **11** can be ascribed to the reactivity of carbonyl groups against the active serine hydroxy group followed by generation of a hemiketal intermediate (a transition state 1), and the facility of N–O bond cleavage (ring opening) followed by generation of an acylated enzyme (a transition state 2). The transition state 1 (TS1) is mainly controlled by electronic properties of the carbonyl carbon atom of compounds **1** and **11**. On the other hand, the transition state 2 (TS2) is chiefly affected by ring strain of each compound. We then calculated strain energies of the four-membered ring,[17,18] the LUMO coefficients and atomic charge of the carbonyl carbon atom of compounds **1** and **11** (Table 3).[19] Table 3 suggests that the ring strain of 2-azetidinone **11** is the same or more favorable than that of 1,3-diazetidion-2,4-dione **1** for increasing reactivity. However, the results of the atomic charge and LUMO coefficients show the opposite tendency. Judging from the potency of compounds **1** and **11**, the electronic property of the carbonyl carbon atom of 1,3-diazetidion-2,4-dione is considered to lead to high activity of 1,3-diazetidion-2,4-dione derivatives against serine proteases, namely, TS1 is speculated to be the rate-determining step in the inhibition reaction.

Conclusion

The present work demonstrates that the 1,3-diazetidine-2,4-dione nucleus is effective as a scaffold for the inhibition of serine proteases.

Table 2. Potency of 1,3-diazetidine-2,4-dione derivatives against seven serine proteases

	IC_{50} (nM)						
Compd	Chymase[a]	α-Chymotrypsin[b]	Cathepsin G[c]	Elastase[d]	Thrombin[e]	Trypsin[f]	Plasmin[g]
1	4.0	0.69	0.39	100	72	39	2900
7	140	2.4	2.3	230	2300	200	na[h]
10	na	740	740	58	5600	39,000	na

[a]Human chymase.
[b]Bovine pancreatic α-chymotrypsin.
[c]Human cathepsin G.
[d]Human neutrophil elastase.
[e]Human thrombin.
[f]Bovine pancreatic trypsin.
[g]Human plasmin.
[h]No activity. $IC_{50} > 10{,}000$ nM.

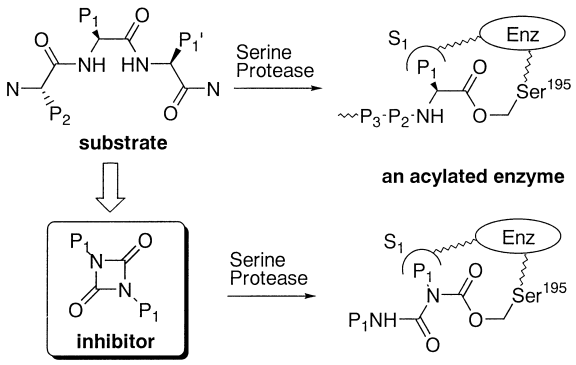
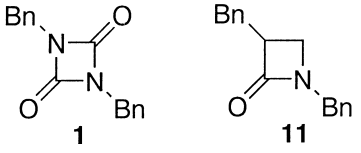


Figure 1.

Table 3. Calculation of ring strain energies, atomic charge, and LUMO coefficients of compounds **1** and **11**



Compd	Strain energy (kcal/mol)	Atomic charge	LUMO coefficients
	PM3/AM1	PM3/AM1	PM3/AM1
1	10.4/33.6	0.303/0.425	−0.0547/−0.583
11	18.1/33.3	0.281/0.321	−0.0049/−0.0049

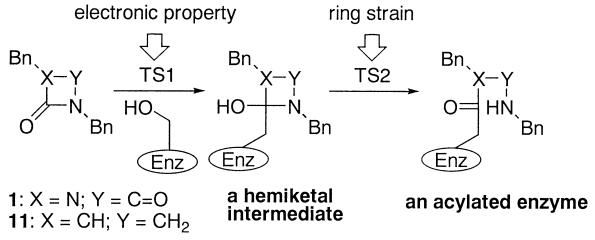


Figure 2.

Acknowledgements

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5. Richter, R.; Ulrich, H. *Synthesis* **1975**, 463.
6. Kuhn, N.; Schwarz, W.; Schmidt, A. *Chem. Ber*. **1977**, *110*, 1130.
7. The structures of compound **1** and **4**–**10** were confirmed by IR, ^{1}H NMR, and mass spectrometric analysis.
8. 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.
9. The inhibitory effects of compounds **1**, **7**, and **10** on the enzymatic activities of seven serine proteases were evaluated using the purified enzymes and chromogenic substrates. The enzymes and substrates used here were as follows: *N*-succinyl-Ala-Ala-Pro-Phe-pNA (Bachem) for bovine pancreatic α-chymotrypsin (Sigma) and human cathepsin G (Wako); Chromozyme TH (Boehringer Mannheim) for human thrombin (Sigma); *N*-succinyl-Ala-Ala-Phe-Arg-pNA (Bachem) for bovine pancreatic trypsin (Sigma); *N*-succinyl-Ala-Ala-Val-pNA (Bachem) for human neutrophil elastase (Athens Research and Technology, Inc.); Chromozym PL (Boehringer Mannheim) for human plasmin (Sigma). The assay buffer used here was as follows: 50 mM Tris–HCl (pH = 8.0) containing 2 mM $CaCl_2$ for α-chymotrypsin, trypsin and elastase; 50 mM Tris–HCl (pH = 7.5) containing 2 mM $CaCl_2$ for cathepsin G; 50 mM Tris–HCl (pH = 7.5) containing 50 mM NaCl for plasmin; 0.1 M Tris–HCl (pH = 8.0) containing 10 mM $CaCl_2$ and 0.1 M NaCl for thrombin.
10. The nomenclature of Schechter and Berger is used to designate the individual amino acid residues (P_2, P_1, P_1', P_2', etc.) of a peptide substrate and the corresponding subsites (S_2, S_1, S_1', S_2', etc.) of the enzyme. The scissile bond is the P_1–P_1' peptide bond. (Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun*. **1967**, *27*, 157).
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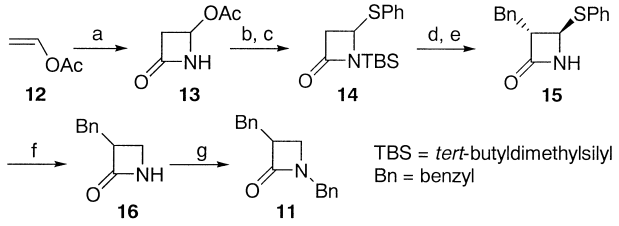
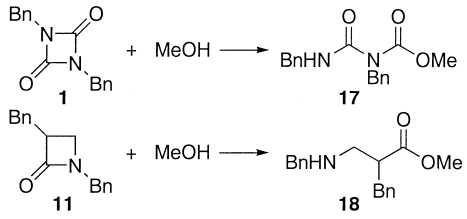
14. 1,2,5-Thiadiazolidin-3-one-1,1-dioxide has been reported as a scaffold for the mechanism-based inhibition of serine proteases. (He, S.; Kuang, R.; Venkataraman, R.; Tu, J.; Truong, T. M.; Chan, H.-K.; Groutas, W. C. *Bioorg. Med. Chem*. **2000**, *8*, 1713).
15. Compound **11** was prepared as follows: (a) ref 20; (b) PhSH, NaOH, H_2O–acetone; (c) TBSCl, Et_3N, CH_2Cl_2 (83% from **13**); (d) BnBr, LDA, THF (87%); (f) *n*-Bu_3SnH, AIBN, PhH (92%); (g) BnBr, NaH, DMF (84%).



16. The chemical stability assay was performed as follows. To 1 mL of sodium methoxide, 10 mM solution in methanol was added 1 μmol of compound **1** or **11** and the solution was stirred at 21 °C. Aliquots were removed at intervals of 2 h, and

analyzed by reverse-phase HPLC. The half-life period ($t_{1/2}$) of compound **1** was 9.5 h. Compound **11** did not react at all with sodium methoxide for 24 h.

17. We assumed that ring strain energies could be approximated by the heat values for the following methanolysis reaction.



18. A theoretical study related to methanolysis of β-lactams has been reported (Massova I.; Kollman, P. A. *J. Phys. Chem. B* **1999**, *103*, 8628).

19. Three-dimensional models of **1** and **11** were constructed based on the published X-ray structure of 1,3-dimethylazetidine-2,4-dione[6] and *N*-benzyl-2-azetidinone derivative (Paquette, L. A.; Rothhaar, R. R.; Isaac, M.; Rogers, L. M.; Rogers, R. D. *J. Org. Chem.* **1998**, *63*, 5463). Low energy conformation, heat of formation, atomic charge and LUMO coefficients of **1** and **11** were calculated by the PM3 and AM1 semiempirical methods as implemented in the MOPAC version 6.0 system. Low energy conformation and heat of formation of **17** and **18** were also calculated by the PM3 and AM1 semiempirical methods.

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