

Discovery and Synthesis of Novel *N*-Cyanopyrazolidine and *N*-Cyanohexahydropyridazine Derivatives as Cathepsin Inhibitors

Jong Hwan Song, Sun Gi Kim, Zae Sung No, Young-Lan Hyun,[†] Dong Ju Jeon,^{*} and Ikyon Kim^{*}

Center for Medicinal Chemistry, Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea

^{*}E-mail: djjeon@kRICT.re.kr; ikyon@kRICT.re.kr

[†]Crystalgenomics, Inc., Seoul 138-736, Korea

Received May 7, 2008

The design, synthesis, and biological evaluation of structurally novel *N*-cyanopyrazolidine and *N*-cyanohexahydropyridazine derivatives as cathepsin inhibitors are described. *In vitro* assay reveals that several compounds exhibit highly potent and selective profiles against cathepsins K or S.

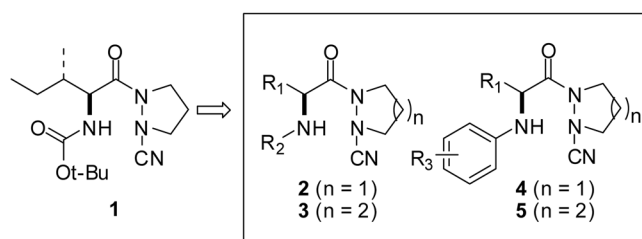
Key Words : Cathepsins, Rheumatoid arthritis, *N*-Cyanopyrazolidine, *N*-Cyanohexahydropyridazine

Introduction

Cathepsins are lysosomal cysteine proteases of the papain family and have been recognized to play a crucial role in a variety of biological processes.¹ For example, cathepsins B, L, and S are implicated in immunological responses² while cathepsin K is a crucial enzyme for bone resorption.³ Thus, investigation of these enzymes as potential drug targets for several diseases such as rheumatoid arthritis, osteoporosis, various types of cancers, stroke, and Alzheimer's disease has been actively pursued. Given the structural homology of cathepsin enzymes, however, selective inhibition of the target cathepsin over other cathepsin family is a prerequisite for further biological evaluation. In the course of our research program directed towards the development of anti-rheumatoid arthritis agents *via* inhibition of cathepsin B, *N*-cyanopyrazolidine compound **1** was identified as a hit as a consequence of HTS utilizing the in-house library. Here we wish to report our design, synthesis, and biological evaluation of *N*-cyanopyrazolidine and *N*-cyanohexahydropyridazine derivatives for selective cathepsin inhibitors.

As described in Figure 1, isoleucine-derived compound **1** exhibits potent and selective inhibition of cathepsin B while displaying no acute toxicity. Based upon these initial data, we decided to investigate the synthesis and biological activity of a series of *N*-cyanopyrazolidines and *N*-cyanohexahydropyridazines for selective cathepsin B inhibitors as shown in Scheme 1. For α -amino acid part, isoleucine, leucine, valine, phenylalanine, and tyrosine were employed.

Synthesis of *N*-cyanopyrazolidine and *N*-cyanohexahydro-



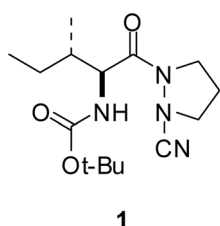
Scheme 1

pyridazine derivatives are outlined in Schemes 2 and 3. Coupling of amino acids **6** with the known pyrazolidine **7** and hexahydropyridazine **8**⁴ in the presence of EDCI and Et₃N afforded the acylated pyrazolidines **9** and hexahydropyridazines **10**, respectively. The resulting amides **9** and **10** were then treated with cyanogen bromide and sodium acetate to give *N*-cyanopyrazolidines **2** and *N*-cyanohexahydropyridazines **3** in good to excellent yields.

For the synthesis of *N*-cyano derivatives bearing *N*-aryl-substituted amino acyl groups, CuI catalyzed *N*-arylation of amino acids was first conducted with various aryl bromides under the Ma's condition⁵ to furnish *N*-arylated amino acids **11**. By following the similar sequence as above, *N*-cyanopyrazolidine and *N*-cyanohexahydropyridazine analogues possessing *N*-arylaminoacyl groups, **4** and **5** were prepared without any event.

In vitro inhibition assays of cathepsins B, L, K, and S with these compounds were conducted.⁶ As shown in Tables 1-4, several compounds were identified to possess a good selectivity profile over these cathepsins.

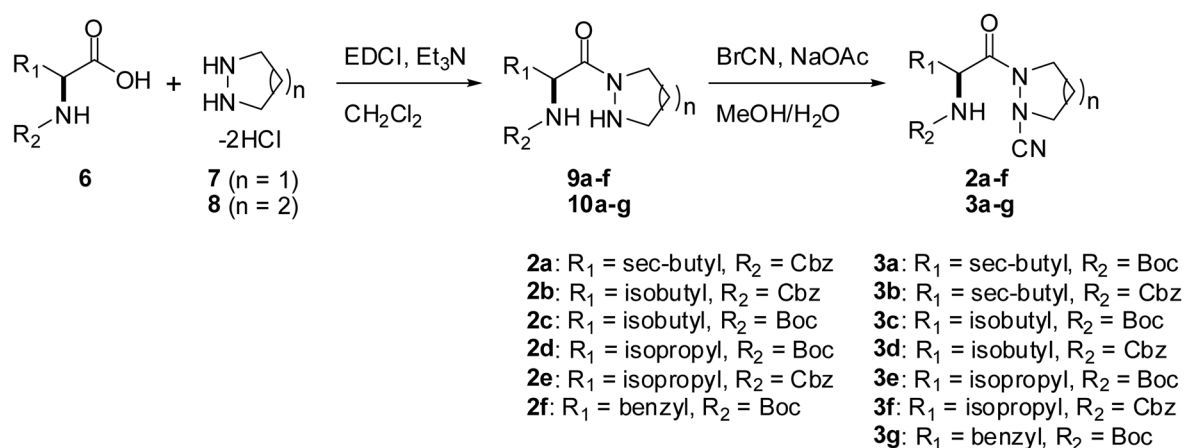
Interestingly, rather cathepsin K- or cathepsin S-selective compounds were discovered. Particularly, both potent inhibition and excellent selectivity against cathepsin K were observed in case of several *N*-cyanohexahydropyridazine derivatives (**3b**, **5f**, and **5j**) whereas compounds such as **2c**, **2f**, **3e**, **4g**, **4l**, and **4m** exhibit great potency and selectivity against cathepsin S. It seemed that hexahydropyridazine ring is better than pyrazolidine for cathepsin K selectivity. As an R₁ part, sterically bulky groups such as benzyl moiety decrease not only selectivity but also potency for cathepsin



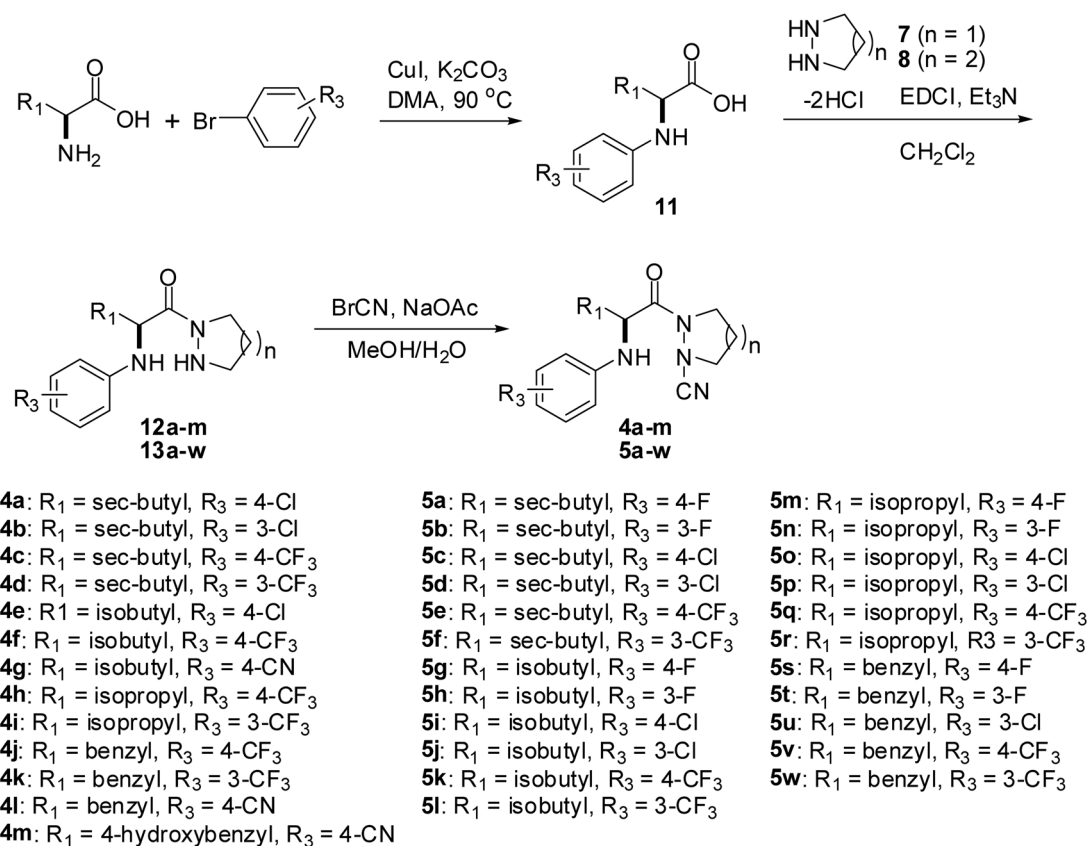
cathepsin K IC₅₀ = 2.60 μ M
 cathepsin L IC₅₀ = 0.80 μ M
 cathepsin B IC₅₀ = 0.01 μ M

Acute toxicity: no toxicity
 - po 1,000 mg/kg
 - ip 500 mg/kg

Figure 1

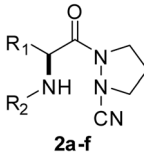


Scheme 2



Scheme 3

Table 1

compounds	IC ₅₀ (μM)				
	Cat B	Cat L	Cat K	Cat S	
	2a	0.049	1.100	>1	0.255
	2b	0.027	0.175	0.007	0.009
	2c	0.680	0.755	–	0.003
	2d	0.037	0.925	0.031	0.023
	2e	0.028	0.095	0.029	0.032
	2f	0.120	>10	0.093	0.004

–: No data available for this compound

Table 2

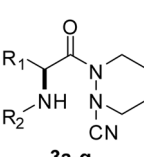
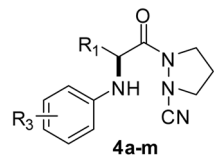
compounds	IC ₅₀ (μM)				
	Cat B	Cat L	Cat K	Cat S	
	3a	0.017	0.185	0.008	0.018
	3b	0.039	0.380	0.001	0.040
	3c	0.021	0.085	0.028	0.004
	3d	0.032	0.180	0.007	0.008
	3e	0.056	0.435	0.055	0.004
	3f	0.011	0.145	0.009	0.031
	3g	0.495	2.150	0.325	0.023

Table 3

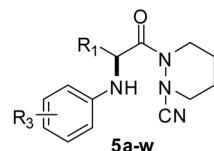
compounds	IC ₅₀ (μM)			
	Cat B	Cat L	Cat K	Cat S
4a	0.037	0.360	>1	0.027
4b	0.015	0.100	0.040	0.005
4c	0.017	0.980	0.170	0.033
4d	0.092	0.046	–	0.031
4e	0.056	0.690	>1	0.013
4f	0.083	0.490	>1	0.007
4g	0.525	1.250	1.000	0.013
4h	0.045	0.470	>1	0.039
4i	0.022	0.100	>1	0.021
4j	0.300	>10	–	0.024
4k	0.053	1.200	>1	0.010
4l	>10	>10	–	0.033
4m	5.100	>10	–	0.037



–: No data available for this compound

Table 4

compounds	IC ₅₀ (μM)			
	Cat B	Cat L	Cat K	Cat S
5a	0.185	0.340	–	0.024
5b	0.135	0.370	–	0.050
5c	0.120	0.343	0.056	0.030
5d	0.043	0.210	0.004	0.007
5e	0.096	0.210	0.024	0.045
5f	0.044	0.046	0.003	0.019
5g	0.052	0.180	0.036	0.016
5h	0.046	0.300	0.042	0.006
5i	0.039	0.150	0.012	0.005
5j	0.028	0.086	0.002	0.016
5k	0.037	0.100	0.015	0.005
5l	0.015	0.032	0.005	0.003
5m	0.056	0.435	0.052	0.035
5n	0.046	0.900	0.135	0.044
5o	0.110	0.610	0.024	0.038
5p	0.032	0.240	0.011	0.029
5q	0.041	0.430	0.040	0.036
5r	0.023	0.048	0.023	0.019
5s	0.180	0.850	–	0.035
5t	0.077	1.300	0.700	0.031
5u	0.053	0.530	0.560	0.016
5v	0.165	0.223	–	0.041
5w	0.042	0.090	0.140	0.007



–: No data available for this compound

K. It should be mentioned that *N*-cyanopyrazolidine ring seemed crucial for cathepsin S selectivity. For selectivity against cathepsin S, isobutyl and isopropyl groups as well as benzyl and 4-hydroxybenzyl groups can be employed for R₁ part, implying more structural flexibility for this region. In addition, cyano group attached to the para position of the phenyl group in the series of **4** (**4g**, **4l**, and **4m**) is conceived to play a role in the selectivity against cathepsin S. Although potency of these derivatives against cathepsin B did not

increase much compared with that of hit compound **1**, two compounds (**4b** and **5n**) having selective inhibitory activity against cathepsins B and S were elected for further biological evaluation.⁷

In conclusion, a series of *N*-cyanopyrazolidine and *N*-cyanohexahydropyridazine compounds were synthesized in search for potent and selective cathepsin B inhibitors. However, contrary to our expectation, several compounds with promising inhibitory activity and selectivity against cathepsins K or S were discovered, respectively. Given the fact that cathepsin K is a good target for curing osteoporosis whereas cathepsin S is an attractive target for various inflammatory diseases, these compounds might be a useful lead for these therapeutic areas. Further studies are ongoing along this line and will be reported in due course.

Experimental Section

General procedure for the synthesis of 9, 10, 12, and 13: To a stirred solution of acid **6** (6 mmol) in dichloromethane (20 mL) at rt were added triethylamine (12 mmol), pyrazolidine **7** (6 mmol), and EDCI (*N*-(3-dimethylamino-propyl)-*N'*-ethylcarbodiimide hydrochloride, 6 mmol) successively. After being stirred at rt for 16 h, the reaction mixture was washed with brine, dried over MgSO₄, and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (hexanes:ethyl acetate) to afford the corresponding amide **9**. Compounds **10**, **12**, and **13** were prepared by following the similar procedure above.

General procedure for the synthesis of 2, 3, 4, and 5: To a stirred solution of amide **9** (1 mmol) in 5 mL of MeOH/H₂O (1:1) were added NaOAc (2 mmol) and CNBr (3 mmol) at rt. After being stirred at rt for 3 h, the reaction mixture was concentrated *in vacuo*. The residue was diluted with CH₂Cl₂ and washed with water. The aqueous layer was extracted with CH₂Cl₂ one more time. The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexanes:ethyl acetate) to give **2**. Compounds **3**, **4**, and **5** were prepared by following the similar procedure above.

2a: 300 MHz ¹H NMR (CDCl₃) δ 7.35-7.29 (m, 5H), 5.49 (m, 1H), 5.08 (m, 2H), 4.98 (m, 1H), 3.60 (m, 1H), 3.46 (m, 1H), 2.06 (m, 2H), 1.75 (m, 1H), 1.50 (m, 1H), 1.12 (m, 1H), 0.94 (d, 3H, *J* = 6.7 Hz), 0.88 (t, 3H, *J* = 7.4 Hz); **2b:** 300 MHz ¹H NMR (CDCl₃) δ 7.66-7.30 (m, 5H), 4.72-4.62 (m, 2H), 3.86-3.82 (m, 1H), 3.68-3.56 (m, 2H), 3.46-3.42 (m, 1H), 2.39-2.33 (m, 2H), 2.02 (m, 2H), 1.70-1.66 (m, 2H), 1.27-1.14 (m, 1H), 0.95 (m, 6H); **2c:** 300 MHz ¹H NMR (CDCl₃) δ 4.95 (d, 1H, *J* = 8.5 Hz), 4.69 (m, 1H), 4.09 (m, 1H), 3.60 (m, 1H), 3.49 (m, 1H), 2.30 (m, 2H), 1.76 (m, 1H), 1.52 (m, 1H), 1.42 (s, 9H), 1.20 (m, 1H), 1.02 (d, 3H, *J* = 6.8 Hz), 0.92 (t, 3H, *J* = 7.5 Hz); **2d:** 300 MHz ¹H NMR (CDCl₃) δ 4.69 (d, 1H, *J* = 9.7 Hz), 4.45 (m, 1H), 3.90 (m, 1H), 3.66-3.55 (m, 2H), 3.34 (m, 1H), 2.36 (m, 2H), 1.41 (s, 9H), 1.08 (d, 3H, *J* = 6.8 Hz), 1.03 (d, 3H, *J* = 6.8 Hz); **2e:** 300 MHz ¹H NMR (CDCl₃) δ 7.38-7.29 (m, 5H), 5.32 (d,

1H, $J = 8.7$ Hz), 4.73 (q, 2H, $J = 20.7$ Hz), 4.79 (m, 1H), 4.12-4.03 (m, 1H), 3.67-3.39 (m, 2H), 2.35-2.14 (m, 2H), 1.06 (d, 3H, $J = 6.6$ Hz), 0.94 (d, 3H, $J = 6.6$ Hz); **3b**: 300 MHz ^1H NMR (CDCl_3) δ 7.34-7.26 (m, 5H), 5.29 (d, 1H, $J = 9.0$ Hz), 5.14-5.02 (m, 2H), 4.81 (m, 1H), 4.51 (d, 1H, $J = 13.2$), 3.55 (m, 2H), 3.19-3.15 (m, 1H), 2.03 (m, 1H), 1.82-1.65 (m, 3H), 1.56-1.49 (m, 1H), 1.32 (m, 1H), 1.01 (d, 3H, $J = 6.6$ Hz), 0.91 (t, 3H, $J = 7.4$ Hz); **3c**: 300 MHz ^1H NMR (CDCl_3) δ 5.25 (d, 1H, $J = 8.7$ Hz), 4.96-4.91 (m, 1H), 4.49 (d, 1H, $J = 13.8$ Hz), 3.53-3.50 (m, 2H), 3.16-3.08 (m, 1H), 1.83-1.68 (m, 4H), 1.52-1.48 (m, 2H), 1.44 (s, 9H), 1.02 (d, 3H, $J = 6.3$ Hz), 0.91 (d, 3H, $J = 6.6$ Hz); **3d**: 300 MHz ^1H NMR (CDCl_3) δ 7.38-7.29 (m, 5H), 5.25 (d, 1H, $J = 8.7$ Hz), 5.14-4.99 (m, 2H), 4.96-4.91 (m, 1H), 4.49 (d, 1H, $J = 13.8$ Hz), 3.53-3.50 (m, 2H), 3.16-3.08 (m, 1H), 1.83-1.68 (m, 4H), 1.52-1.48 (m, 2H), 1.02 (d, 3H, $J = 6.3$ Hz), 0.91 (d, 3H, $J = 6.6$ Hz); **3e**: 300 MHz ^1H NMR (CDCl_3) δ 5.03-5.00 (d, 1H, $J = 9.0$ Hz), 4.73-4.68 (m, 1H), 4.53-4.49 (d, 1H, $J = 9.9$ Hz), 3.60-3.52 (m, 2H), 3.18-3.10 (m, 1H), 2.13-2.01 (m, 2H), 1.99-1.84 (m, 2H), 1.80 (m, 1H), 1.43 (s, 9H), 1.03-1.01 (d, 3H, $J = 6.6$ Hz), 0.93 (d, 3H, $J = 6.9$ Hz); **3f**: 300 MHz ^1H NMR (CDCl_3) δ 7.36-7.34 (m, 5H), 5.28 (d, 1H, $J = 9.6$ Hz), 5.15-5.03 (m, 2H), 4.78-4.75 (m, 1H), 4.51 (d, 1H, $J = 12.0$ Hz), 3.53-3.50 (m, 2H), 3.20-3.11 (m, 1H), 2.06-1.99 (m, 2H), 1.83-1.79 (m, 1H), 1.05-1.03 (d, 3H, $J = 6.6$ Hz), 0.93 (d, 3H, $J = 6.9$ Hz); **3g**: 300 MHz ^1H NMR (CDCl_3) δ 7.32-7.18 (m, 5H), 5.09-5.05 (m, 2H), 4.51-4.47 (m, 1H), 3.53-3.50 (d, 1H, $J = 9.0$ Hz), 3.53-3.50 (m, 2H), 3.20-3.11 (m, 1H), 3.17-3.08 (m, 4H), 3.06-2.98 (m, 1H), 2.80-2.03 (m, 1H), 1.37 (s, 9H); **4a**: 300 MHz ^1H NMR (CDCl_3) δ 7.10 (d, 2H, $J = 8.7$ Hz), 6.60 (d, 2H, $J = 9.0$ Hz), 4.40-4.34 (m, 1H), 4.11 (d, 1H, $J = 10.5$ Hz), 3.89 (m, 1H), 3.58 (m, 2H), 3.23 (m, 2H), 3.22 (m, 1H), 2.25 (m, 2H), 1.88 (m, 2H), 1.33-1.17 (m, 1H), 1.03 (d, 3H, $J = 7.2$ Hz), 0.94 (t, 3H, $J = 7.2$ Hz); **4b**: 300 MHz ^1H NMR (CDCl_3) δ 7.05 (t, 1H, $J = 7.8$ Hz), 6.68 (d, 1H, $J = 8.1$ Hz), 6.61 (s, 1H), 6.54 (d, 1H, $J = 8.1$ Hz), 4.41 (m, 1H), 4.21 (d, 1H, $J = 10.8$ Hz), 3.95 (m, 1H), 3.58 (m, 2H), 3.30 (m, 1H), 2.21 (m, 2H), 1.75 (m, 2H), 1.29 (m, 1H), 1.05 (d, 3H, $J = 6.9$ Hz), 0.94 (t, 3H, $J = 7.5$ Hz); **4c**: 300 MHz ^1H NMR (CDCl_3) δ 7.32 (d, 2H, $J = 8.5$ Hz), 6.61 (d, 2H, $J = 8.5$ Hz), 4.42-4.34 (m, 2H), 3.89-3.79 (m, 1H), 3.59-3.43 (m, 2H), 3.27-3.18 (m, 1H), 2.32-2.15 (m, 2H), 1.85-1.58 (m, 2H), 1.25-1.12 (m, 1H), 0.98 (d, 3H, $J = 6.8$ Hz), 0.87 (t, 3H, $J = 7.4$ Hz); **4d**: 300 MHz ^1H NMR (CDCl_3) δ 7.26-7.21 (m, 1H), 6.96 (d, 1H, $J = 7.5$ Hz), 6.83-6.81 (m, 2H), 4.47 (dd, 2H, $J = 10.5, 6.9$ Hz), 4.33 (d, 1H, $J = 10.5$ Hz), 3.99-3.90 (m, 1H), 3.61-3.52 (m, 2H), 3.29-3.20 (m, 1H), 2.38-2.20 (m, 2H), 1.90-1.68 (m, 2H), 1.33-1.24 (m, 1H), 1.07 (d, 3H, $J = 6.8$ Hz), 0.95 (t, 3H, $J = 7.4$ Hz); **4e**: 300 MHz ^1H NMR (CDCl_3) δ 7.10 (d, 2H, $J = 8.7$ Hz), 6.56 (d, 2H, $J = 8.7$ Hz), 4.65-4.56 (m, 1H), 4.08 (d, 1H, $J = 10.5$ Hz), 3.91-3.79 (m, 2H), 1.96-1.81 (m, 1H), 1.69-1.50 (m, 2H), 1.04-0.98 (m, 6H); **4f**: 300 MHz ^1H NMR (CDCl_3) δ 7.49 (d, 2H, $J = 8.7$ Hz), 6.64 (d, 2H, $J = 8.7$ Hz), 4.73-4.69 (m, 1H), 4.39 (d, 1H, $J = 9.9$ Hz), 3.88-3.85 (m, 1H), 3.84-3.54 (m, 2H), 3.43-3.39 (m, 1H), 2.37-2.30 (m, 2H), 1.67-1.65 (m, 1H), 1.64-1.55 (m, 2H), 1.02

(m, 6H); **4g**: 300 MHz ^1H NMR (CDCl_3) δ 7.42 (d, 2H, $J = 8.7$ Hz), 6.59 (d, 2H, $J = 8.7$ Hz), 4.72-4.62 (m, 2H), 3.86-3.82 (1H, m), 3.68-3.56 (m, 2H), 3.46-3.42 (m, 1H), 2.39-2.33 (m, 2H), 1.70-1.66 (m, 1H), 1.27-1.14 (m, 2H), 0.95 (m, 6H); **4h**: 300 MHz ^1H NMR (CDCl_3) δ 7.38 (d, 2H, $J = 8.4$ Hz), 6.69 (d, 2H, $J = 8.4$ Hz), 4.55 (d, 1H, $J = 10.2$ Hz), 4.47-4.42 (m, 1H), 3.89-3.88 (m, 1H), 3.63-3.53 (m, 2H), 3.34-3.30 (m, 1H), 2.35-2.28 (m, 2H), 2.14-2.10 (m, 1H), 1.07 (m, 6H); **4i**: 300 MHz ^1H NMR (CDCl_3) δ 7.26-7.21 (m, 1H), 6.96 (d, 1H, $J = 7.5$ Hz), 6.83-6.81 (m, 2H), 4.47-4.37 (m, 2H), 3.96-3.93 (m, 1H), 3.60-3.55 (m, 1H), 3.29-3.25 (m, 1H), 2.27-2.09 (m, 2H), 1.10 (m, 6H); **4j**: 300 MHz ^1H NMR (CDCl_3) δ 7.32 (d, 2H, $J = 8.7$ Hz), 7.25-7.14 (m, 1H), 4.58 (d, 1H, $J = 9.9$ Hz), 3.62-3.57 (m, 2H), 3.20-3.12 (m, 1H), 3.02 (d, 2H, $J = 6.9$ Hz), 2.60 (m, 1H), 2.13-2.00 (m, 2H); **4k**: 300 MHz ^1H NMR (CDCl_3) δ 7.34-7.22 (m, 6H), 6.97 (d, 1H, $J = 7.5$ Hz), 6.80-6.77 (m, 2H), 4.93-4.88 (m, 1H), 4.48 (d, 1H, $J = 9.9$ Hz), 3.71-3.62 (m, 2H), 3.29-3.21 (m, 1H), 3.12-3.08 (m, 2H), 2.88-2.85 (m, 1H), 2.24-2.15 (m, 2H); **4l**: 300 MHz ^1H NMR (CDCl_3) δ 7.42 (d, 2H, $J = 8.7$ Hz), 7.33-7.20 (m, 5H), 6.60 (d, 2H, $J = 9.0$ Hz), 4.93-4.91 (m, 1H), 4.83 (d, 1H, $J = 9.3$ Hz), 3.71-3.67 (m, 2H), 3.31-3.23 (m, 1H), 3.10 (d, 2H, $J = 6.9$ Hz), 2.73 (m, 1H), 2.24-2.14 (m, 2H); **4m**: 300 MHz ^1H NMR (CDCl_3) δ 7.39 (d, 2H, $J = 8.1$ Hz), 7.03 (d, 2H, $J = 8.1$ Hz), 6.74 (d, 2H, $J = 8.1$ Hz), 6.58 (d, 2H, $J = 8.1$ Hz), 4.92-4.89 (m, 2H), 3.69-3.35 (m, 2H), 3.33-3.27 (m, 1H), 3.02-3.01 (m, 2H), 2.96-2.90 (m, 1H), 2.25-2.19 (m, 2H); **5a**: 300 MHz ^1H NMR (CDCl_3) δ 6.91-6.82 (m, 2H), 6.68-6.63 (m, 2H), 4.54-4.49 (m, 1H), 4.34 (m, 1H), 3.98 (m, 1H), 3.55-3.40 (m, 1H), 3.15-2.74 (m, 2H), 2.02-1.57 (m, 5H), 1.28-1.22 (m, 2H), 1.01 (d, 3H, $J = 6.6$ Hz), 0.94 (t, 3H, $J = 7.2$ Hz); **5b**: 300 MHz ^1H NMR (CDCl_3) δ 7.13-7.05 (m, 1H), 6.45-6.34 (m, 3H), 4.55-4.44 (m, 2H), 4.26 (d, 1H, $J = 9.9$ Hz), 3.55-3.50 (m, 1H), 3.18-2.98 (m, 2H), 2.11-1.97 (m, 1H), 1.83-1.57 (m, 4H), 1.26-1.15 (m, 2H), 1.02 (d, 3H, $J = 6.6$ Hz), 0.93 (t, 3H, $J = 7.5$ Hz); **5c**: 300 MHz ^1H NMR (CDCl_3) δ 7.11 (d, 2H, $J = 6.9$ Hz), 6.62 (d, 2H, $J = 6.9$ Hz), 4.54-4.35 (m, 2H), 4.13 (m, 1H), 3.59-3.44 (m, 1H), 3.22-2.89 (m, 2H), 2.06-1.95 (m, 1H), 1.82-1.54 (m, 5H), 1.26-1.19 (m, 1H), 1.01 (d, 3H, $J = 6.6$ Hz), 0.95 (t, 3H, $J = 7.2$ Hz); **5d**: 300 MHz ^1H NMR (CDCl_3) δ 7.07 (t, 1H, $J = 8.1$ Hz), 6.70-6.64 (m, 2H), 6.55 (d, 1H, $J = 9.6$ Hz), 3.55-3.52 (m, 1H), 4.49 (m, 2H), 4.22 (d, 1H, $J = 9.6$ Hz), 3.53 (m, 1H), 3.15 (m, 2H), 2.10-1.99 (m, 1H), 1.75 (m, 5H), 1.24 (m, 1H), 1.02 (d, 3H, $J = 6.6$ Hz), 0.93 (t, 3H, $J = 7.5$ Hz); **5e**: 300 MHz ^1H NMR (CDCl_3) δ 7.40 (d, 2H, $J = 8.6$ Hz), 6.68 (d, 2H, $J = 8.2$ Hz), 4.57-4.45 (m, 3H), 3.60-3.49 (m, 1H), 3.23-2.99 (m, 2H), 2.09-2.00 (m, 1H), 1.88-1.56 (m, 5H), 1.26-1.15 (m, 1H), 1.02 (d, 3H, $J = 6.7$ Hz), 0.93 (t, 3H, $J = 7.4$ Hz); **5f**: 300 MHz ^1H NMR (CDCl_3) δ 7.29-7.23 (m, 1H), 6.97 (d, 1H, $J = 7.2$ Hz), 6.88-6.82 (m, 2H), 4.54-4.34 (m, 3H), 3.56-3.51 (m, 1H), 3.23-2.97 (m, 2H), 2.06-2.02 (m, 1H), 1.83-1.51 (m, 4H), 1.30-1.17 (m, 2H), 1.05 (d, 3H, $J = 6.9$ Hz), 0.94 (t, 3H, $J = 7.2$ Hz); **5g**: 300 MHz ^1H NMR (CDCl_3) δ 6.91-6.83 (m, 2H), 6.64-6.59 (m, 2H), 4.61-4.47 (m, 2H), 3.92 (m, 1H), 3.62-3.52 (m, 1H), 3.29-3.06 (m,

2H), 2.06-2.02 (m, 1H), 1.90-1.75 (m, 4H), 1.60-1.56 (m, 2H), 1.04-0.95 (m, 6H); **5h**: 300 MHz ^1H NMR (CDCl_3) δ 7.14-7.06 (m, 1H), 6.45-6.29 (m, 3H), 4.71-4.49 (m, 2H), 4.21 (d, 1H, $J = 10.5$ Hz), 3.62-3.57 (m, 1H), 3.26-3.09 (m, 2H), 2.09-2.00 (m, 1H), 1.90-1.82 (m, 3H), 1.62-1.58 (m, 3H), 1.04-0.95 (m, 6H); **5i**: 300 MHz ^1H NMR (CDCl_3) δ 7.12 (d, 2H, $J = 8.7$ Hz), 6.58 (d, 2H, $J = 8.4$ Hz), 4.67-4.48 (m, 2H), 4.08 (m, 1H), 3.59-3.54 (m, 1H), 3.26-3.11 (m, 2H), 2.12-1.98 (m, 1H), 1.90-1.81 (m, 3H), 1.61-1.57 (m, 3H), 1.03-0.98 (m, 3H); **5j**: 300 MHz ^1H NMR (CDCl_3) δ 7.08 (t, 1H, $J = 7.8$ Hz), 6.70 (d, 1H, $J = 7.8$ Hz), 6.58 (s, 1H), 6.53 (d, 1H, $J = 8.1$ Hz), 4.70-4.59 (m, 1H), 4.51 (d, 1H, $J = 11.7$ Hz), 4.1 (m, 1H), 3.61 (m, 1H), 3.18 (m, 2H), 2.13-2.01 (m, 1H), 1.89 (m, 3H), 1.67-1.57 (m, 3H), 1.04-0.98 (m, 6H); **5k**: 300 MHz ^1H NMR (CDCl_3) δ 7.41 (d, 2H, $J = 8.7$ Hz), 6.69-6.63 (m, 2H), 4.77-4.66 (m, 1H), 4.54-4.44 (m, 2H), 3.62-3.58 (m, 1H), 3.26-3.09 (m, 2H), 2.10-2.00 (m, 1H), 1.92-1.80 (m, 3H), 1.68-1.61 (m, 3H), 1.03-0.97 (m, 6H); **5l**: 300 MHz ^1H NMR (CDCl_3) δ 7.29-7.23 (m, 1H), 6.99-6.93 (d, 1H, $J = 7.8$ Hz), 6.82-6.76 (m, 2H), 4.77-4.67 (m, 1H), 4.53-4.49 (d, 1H, $J = 11.7$ Hz), 4.29 (m, 1H), 3.64-3.59 (m, 1H), 3.29-3.09 (m, 2H), 2.09-2.05 (m, 1H), 1.94-1.83 (m, 3H), 1.64-1.60 (m, 3H), 1.05-0.96 (m, 6H); **5m**: 300 MHz ^1H NMR (CDCl_3) δ 6.91-6.85 (m, 2H), 6.68-6.64 (m, 2H), 4.53-4.49 (m, 1H), 4.30 (m, 1H), 4.04 (m, 1H), 3.47-3.43 (m, 1H), 3.15-2.80 (m, 2H), 2.07-2.00 (m, 1H), 1.80-1.68 (m, 2H), 1.57-1.45 (m, 2H), 1.07-0.99 (m, 6H); **5n**: 300 MHz ^1H NMR (CDCl_3) δ 7.13-7.05 (m, 1H), 6.46-6.35 (m, 3H), 4.54-4.31 (m, 3H), 3.56-3.51 (m, 1H), 3.18-3.01 (m, 2H), 2.13-1.98 (m, 2H), 1.83-1.73 (m, 2H), 1.66-1.54 (m, 1H), 1.06 (d, 3H, $J = 6.9$ Hz), 1.01 (d, 3H, $J = 6.9$ Hz); **5o**: 300 MHz ^1H NMR (CDCl_3) δ 7.12 (d, 2H, $J = 9.0$ Hz), 6.63 (d, 2H, $J = 9.0$ Hz), 4.53-4.48 (m, 1H), 4.39-4.32 (m, 1H), 4.20-4.19 (m, 1H), 3.60-3.48 (m, 1H), 3.27-2.92 (m, 2H), 2.11-2.01 (m, 1H), 1.83-1.72 (m, 2H), 1.59-1.51 (m, 2H), 1.06 (d, 3H, $J = 6.9$ Hz), 1.01 (d, 3H, $J = 6.6$ Hz); **5p**: 300 MHz ^1H NMR (CDCl_3) δ 7.07 (t, 1H, $J = 8.1$ Hz), 6.70-6.50 (m, 2H), 6.56 (d, 1H, $J = 8.1$ Hz), 4.54-4.40 (m, 2H), 4.29 (d, 1H, $J = 10.2$ Hz), 3.54 (m, 1H), 3.18-3.03 (m, 2H), 2.12-2.02 (m, 2H), 1.83-1.74 (m, 2H), 1.65 (m, 1H), 1.06 (d, 3H, $J = 6.6$ Hz), 1.01 (d, 3H, $J = 6.9$ Hz); **5q**: 300 MHz ^1H NMR (CDCl_3) δ 7.33 (d, 2H, $J = 8.4$ Hz), 6.66 (d, 2H, $J = 7.8$ Hz), 4.468 (m, 3H), 3.57-3.45 (m, 1H), 3.17-2.95 (m, 2H), 2.10-1.97 (m, 2H), 1.86-1.69 (m, 2H), 1.52 (m, 1H), 1.00 (d, 3H, $J = 6.6$ Hz), 0.93 (d, 3H, $J = 6.6$ Hz); **5r**: 300 MHz ^1H NMR (CDCl_3) δ 7.26 (t, 1H, $J = 7.8$ Hz), 6.96 (d, 1H, $J = 7.5$ Hz), 6.85-6.83 (m, 2H), 4.53-4.42 (m, 3H), 3.56-3.52 (m, 1H), 3.18-3.01 (m, 2H), 2.17-1.95 (m, 2H), 1.83-1.73 (m, 2H), 1.56-1.52 (m, 1H), 1.09 (d, 3H, $J = 6.9$ Hz), 1.02 (d, 3H, $J = 6.6$ Hz); **5s**: 300 MHz ^1H NMR (CDCl_3) δ 7.33-7.22 (m, 5H), 6.93-6.83 (m, 2H), 6.70-6.66 (m, 1H), 6.57-6.54 (m, 1H), 4.90-4.78 (m, 1H), 4.52-4.42 (m, 1H), 4.12 (d, 1H, $J = 6.9$ Hz), 3.16-2.94 (m, 5H), 1.84-1.67 (m, 2H), 1.58-1.41 (m, 2H); **5t**: 300 MHz ^1H NMR (CDCl_3) δ 7.35-7.24 (m, 5H), 7.16-7.06 (m, 1H), 6.50-6.23

(m, 3H), 4.94-4.82 (m, 1H), 4.53-4.33 (m, 2H), 3.19-2.95 (m, 4H), 1.86-1.33 (m, 5H); **5u**: 300 MHz ^1H NMR (CDCl_3) δ 7.35-7.23 (m, 5H), 7.13-7.01 (m, 1H), 6.73-6.44 (m, 3H), 4.90-4.86 (m, 1H), 4.49-4.30 (m, 2H), 3.25-2.91 (m, 4H), 2.08-1.33 (m, 5H); **5v**: 300 MHz ^1H NMR (CDCl_3) δ 7.44-7.18 (m, 7H), 6.72 (d, 1H, $J = 8.4$ Hz), 6.57 (d, 1H, $J = 8.1$ Hz), 4.99-4.92 (m, 1H), 4.68-4.46 (m, 2H), 3.22-2.93 (m, 4H), 2.17-2.1 (m, 1H), 1.88-1.46 (m, 4H); **5w**: 300 MHz ^1H NMR (CDCl_3) δ 7.33-7.20 (m, 6H), 7.00-6.85 (m, 2H), 6.74-6.69 (m, 1H), 4.97-4.91 (m, 1H), 4.54-4.47 (m, 2H), 3.25-2.95 (m, 4H), 1.88-1.47 (m, 5H).

Acknowledgments. We thank the Center for Biological Modulators and Korea Research Institute of Chemical Technology for generous financial support. We also thank Dr. Seong Hwan Kim for helpful discussions.

References and Notes

- (a) McGrath, M. E. *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *28*, 181. (b) Turk, V.; Turk, B.; Turk, D. *EMBO J.* **2001**, *20*, 4629. (c) Leung-Toung, R.; Li, W.; Tam, T. F.; Karimian, K. *Curr. Med. Chem.* **2002**, *9*, 979. (d) Lecaille, F.; Kaleta, J.; Brömme, D. *Chem. Rev.* **2002**, *102*, 4459.
- (a) Turk, B.; Turk, D.; Turk, V. *Biochim. Biophys. Acta* **2000**, *1477*(1-2), 98. (b) Buhling, F.; Fengler, A.; Brandt, W.; Welte, T.; Ansoorge, S.; Nagler, D. K. *Adv. Exp. Med. Biol.* **2000**, *477*, 241.
- (a) Brömme, D.; Okamoto, K. *Biol. Chem. Hoppe-Seyler* **1995**, *376*, 379. (b) Drake, F. H.; Dodds, R. A.; James, I. A.; Connor, J. R.; Debouck, C.; Richardson, S.; Lee-Rykaczewski, L.; Coleman, L.; Riemann, D.; Barthlow, R.; Hastings, G.; Gowen, M. *J. Biol. Chem.* **1996**, *271*, 12511. (c) Gelb, B. D.; Shi, G.-P.; Chapman, H. A.; Desnick, R. J. *Science* **1996**, *273*, 1236. (d) Saftig, P.; Hunziker, E.; Wehmeyer, O.; Jones, S.; Boyde, A.; Rommelskirch, W.; Moritz, J. D.; Schu, P.; von Figura, K. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13453.
- (a) Boros, E. E.; Bouvier, F.; Randhawa, S.; Rabinowitz, M. H. *J. Heterocycl. Chem.* **2001**, *38*, 613. (b) Ahn, J. H.; Kim, J. A.; Kim, H.-M.; Kwon, H.-M.; Huh, S.-C.; Rhee, S. D.; Kim, K. R.; Yang, S.-D.; Park, S.-D.; Lee, J. M.; Kim, S. S.; Cheon, H. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1337. (c) Ahn, J. H.; Shin, M. S.; Jun, M. A.; Jung, S. H.; Kang, S. K.; Kim, K. R.; Rhee, S. D.; Kang, N. S.; Kim, S. Y.; Sohn, S.-K.; Kim, S. G.; Jin, M. S.; Lee, J. O.; Cheon, H. G.; Kim, S. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2622.
- Ma, D.; Zhang, Y.; Yao, J.; Wu, S.; Tao, F. *J. Am. Chem. Soc.* **1998**, *120*, 12459.
- In vitro* enzymatic activity assay for cathepsins B, L, S, and K: The enzymatic reaction was performed in a 96-well plate (Costar) by mixing reaction buffer (100 mM NaOAc, 2 mM EDTA, 3 mM DTT, pH 5.5), 5 μL of 400 μM substrate (Z-RR-PNA; biomol), 5.88 nM recombinant human cathepsin B (1-339 amino acid) and 5% (v/v) compound (12.5 mM DMSO stock solution used). The mixture was incubated at 30 $^\circ\text{C}$ for 2 h and then its absorbance was measured at 405 nm in Benchmark plus (Bio-Rad). The substrate Z-FR-pNA (biomol) was used in assay for cathepsins L and S, and Z-GPR-AMC was used for cathepsin K. In addition, 300 mU recombinant cathepsin L (Calbiochem), 100 nM recombinant human cathepsin S (1-331 amino acid), 20 nM recombinant human cathepsin K (1-329 amino acid) were used in each assay.
- In vivo* efficacy test of these in CIA (collagen induced arthritis) murine model will be discussed elsewhere. Trentham, D. E.; Townes, A. S.; Kang, A. H. *J. Exp. Med.* **1977**, *146*, 857.