

Synthesis and biological evaluation of chromone carboxamides as calpain inhibitors

Kwang Seob Lee,^{a,b} Seon Hee Seo,^a Yong Ha Lee,^a Ha Dong Kim,^c Moon Ho Son,^c Bong Young Chung,^b Jae Yeol Lee,^d Changbae Jin^a and Yong Sup Lee^{e,*}

^aLife Sciences Division, Korea Institute of Science & Technology, PO Box 131 Cheongryang, Seoul 130-650, Republic of Korea

^bDepartment of Chemistry, Korea University, 1-Anamdong, Seoul 136-701, Republic of Korea

^cDepartment of Pharmacology, Research Laboratories, 47-5, Dong-A Pharm. Co., Ltd, Yongin-Si 449-900, South Korea

^dResearch Institute of Basic Science and Department of Chemistry, Kyung Hee University, 1 Hoegi-dong, Dongdaemoon-ku, Seoul 130-701, Republic of Korea

^eKyung Hee East-West Pharmaceutical Research Institute and Department of Pharmaceutical Science, College of Pharmacy, Kyung Hee University, 1 Hoegi-dong, Dongdaemoon-ku, Seoul 130-701, Republic of Korea

Received 28 January 2005; accepted 23 March 2005

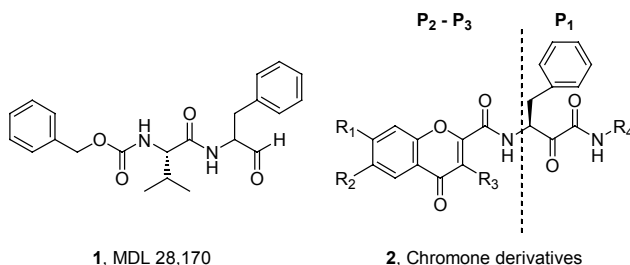
Available online 25 April 2005

Abstract—Excessive calpain activations contribute to serious cellular damage and have been found in many pathological conditions. Novel chromone carboxamides derived from ketoamides were prepared and evaluated for μ -calpain inhibition. Among synthesized, compound **2i** was the most potent calpain inhibitor with an IC_{50} value of $0.24 \pm 0.11 \mu M$ comparable to the activity of peptide aldehyde calpain inhibitor MDL 28,170. Furthermore, compound **2i** showed higher selectivity for μ -calpain over two related cysteine proteases cathepsin B and cathepsin L, suggesting the chromone ring as a good scaffold for selective μ -calpain inhibitors.

© 2005 Elsevier Ltd. All rights reserved.

Calpains are calcium-dependent, intracellular proteolytic enzymes and found in many cells. Calpains are referred to as cysteine proteases because they include a cysteine residue in the catalytic process.¹ Two major forms of calpains have been identified: calpain I (or μ -calpain) and calpain II (or m-calpain), which require micromolar and millimolar concentrations of calcium ions for activation, respectively.² Excessive calpain activation contributes to serious cellular damage or even cell death and has been found in a number of pathological conditions, for example, cerebral ischemia,³ myocardial infarction,⁴ traumatic brain injury,⁵ Alzheimer's disease,⁶ cataract of eyes,⁷ and inflammation⁸ to implicate that these diseases are presumably associated with elevated intracellular calcium concentrations. For this reason, considerable efforts have been focused on the design and synthesis of calpain inhibitors as a novel therapeutic principle for many years.

Many reversible and irreversible calpain inhibitors have been reported. Most of them are derived from peptides, in particular dipeptide aldehydes such as Z-Val-Phe-H (**1**, MDL 28,170).⁹ However, MDL 28,170 suffers from some disadvantage such as nonselectivity, instability during storage and excessive metabolism owing to the peptide character and high reactivity of aldehyde moiety present.¹⁰ Therefore, the recent studies are mainly focused on the design of less peptide-like calpain inhibitors to lead piperidine carboxamides,¹¹ benzoyl ketoamides,¹² and peptidyl hydrazones.¹³



Keywords: Calpain; Inhibitor; Chromone; Ischemia; Stroke.

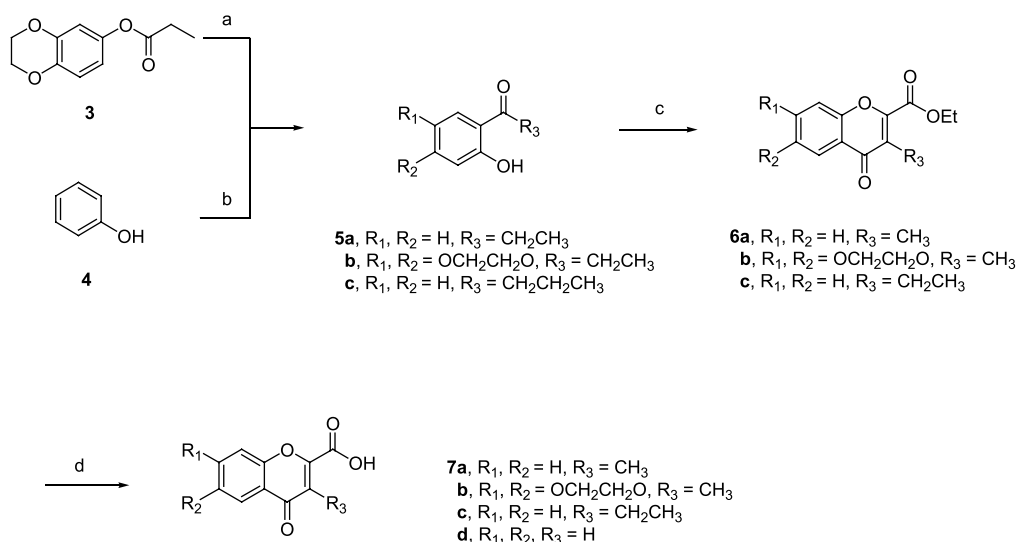
* Corresponding author. Tel.: +82 2 961 0370; fax: +82 2 966 3885; e-mail: kyslee@khu.ac.kr

Likewise, in our attempts for search of novel inhibitors, chromones were selected as new building blocks in the P₂–P₃ region of the enzyme and as a replacement of α -amino acid moiety, Z-Val within MDL 28,170 to reduce peptide character. We envisioned that the pyran ring having a R₃-substituent in compound **2** can be considered as a cyclic analogue of valine moiety in the structure of MDL 28,170. Furthermore, it is anticipated that additional hydrogen bonds may be possible via interaction of carbonyl oxygen of pyran ring with calpain residues. The ketoamide was also used as a warhead in the new inhibitors since several ketoamide-derived inhibitors showed improved metabolic stability in vitro and in vivo.¹⁴ Herein we describe the synthesis of chromone derivatives **2** as novel calpain inhibitors and their biological evaluation for μ -calpain inhibition. To investigate the influence of substituents on inhibitory effect, we set the variations at chromone ring (R₁, R₂, and R₃) and amide part (R₄) in the design of inhibitors.

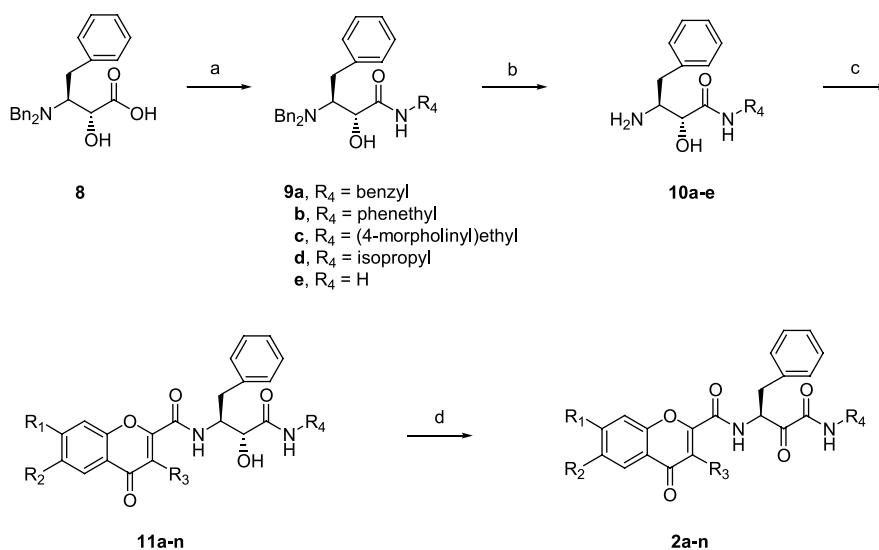
Chromone derivatives **2** were synthesized by coupling of chromone carboxylic acids **7** with amino alcohols **10** followed by oxidation of hydroxy group as shown in Scheme 2. Synthetic route to compounds **7** is detailed in Scheme 1. 2-Hydroxypropiophenone (**5a**) and chromone-2-carboxylic acid (**7d**) were purchased, and 2-hydroxybutyrophenone (**5c**) was prepared from phenol (**4**) by Friedel–Craft acylation with butyryl chloride. Dioxane-fused propiophenone (**5b**) was prepared by Fries rearrangement of compound **3**,¹⁵ which was obtained from benzodioxane via two-step sequence; Friedel–Craft acylation with propionyl chloride and Bayer–Villiger oxidation.¹⁶ Chromone rings were constructed by acylation of 2-hydroxyphenones **5** with ethyl chlorooxoacetate followed by in situ cyclization of the resulting esters in the presence of pyridine to provide compounds **6**.¹⁷ The ethyl ester groups in compounds **6** were finally hydrolyzed with KOH to yield chromone carboxylic acids **7**.

The P₁ building blocks 3-amino-2-hydroxybutanoic acid amides (**10**) were prepared from 3-dibenzylamino-2-hydroxy acid (**8**)¹⁸ as illustrated in Scheme 2. Compound **8** was coupled to various amines using EDC/HOBt and then debenzylated by hydrogenolysis under atmospheric pressure of hydrogen with palladium hydroxide to provide amino alcohols **10**. Compounds **10** were coupled to chromone-2-carboxylic acid derivatives **7** using again EDC/HOBt to yield compounds **11**, which subsequently were transformed into the chromone derivatives **2** by oxidation under Dess–Martin periodinane conditions.¹⁸

The biological activities of the synthesized compounds were evaluated for inhibition of μ -calpain using a casein-Coomassie blue microplate assay according to the method previously reported.¹⁹ The percent inhibition of cathepsin B and cathepsin L was tested at three concentrations according to the procedure described in the literature using Cbz-Arg-Arg-pNA for cathepsin B²⁰ and Cbz-Phe-Arg-AMC for cathepsin L²¹ as a substrate. The results are summarized in Table 1. MDL 28,170 (**1**) is well known as the most potent calpain inhibitor and therefore its biological activities were also tested in our assay systems for comparison. First, we examined the importance of a chiral center in chromone derivatives for inhibitory activity by synthesizing **2a** and its antipode (structure not shown) starting from L-phenylalanine and D-phenylalanine, respectively, by the same method. The inhibitory effect of **2a** (IC₅₀ = 0.72 $\pm$ 0.17 μ M) with *S*-configuration on μ -calpain was superior to that of its antipode (IC₅₀ = 8.32 $\pm$ 0.38 μ M) and therefore all chromone derivatives were prepared from L-phenylalanine. Second, the introduction of dioxane ring in the chromone ring generally resulted in the decreased inhibitory activity of the parent compound irrespective of amide substituents R₄. However, amide substituents were also important in the activity. Compounds **2a** and **c** possessing benzyl and phenethyl amide

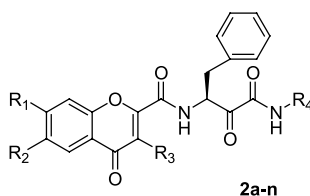


Scheme 1. Reagents and conditions: (a) TiCl₄, 1,2-dichloroethane, rt; (b) butyryl chloride, AlCl₃, CS₂, rt; (c) ethyl chlorooxoacetate, pyridine, 60 °C $\rightarrow$ reflux; (d) KOH, EtOH/H₂O, rt.



Scheme 2. Reagents and conditions: (a) R_4 -NH₂, EDC, HOBT, CH₂Cl₂/THF, rt; (b) H₂, Pd(OH)₂/C, MeOH, rt; (c) **7a-d**, EDC, HOBT, CH₂Cl₂/THF, rt; (d) Dess–Martin periodinane, CH₂Cl₂/THF, 0 °C → rt.

Table 1. Synthesized chromone derivatives and their activities in inhibition of μ -calpain, cathepsin B, and cathepsin L



Compd	R_1, R_2	R_3	R_4	μ -Calpain ^a inhibition IC ₅₀ (μ M) ^d	% Inhibition of cathepsin B ^b at concn of			% Inhibition of cathepsin L ^c at concn of		
					0.2 μ M	2 μ M	20 μ M	0.2 μ M	2 μ M	20 μ M
2a	H, H	Methyl	Benzyl	0.72 $\pm$ 0.15	0.0	21.3	67.9	8.1	65.3	95.6
2b	–OCH ₂ CH ₂ –	Methyl	Benzyl	2.48 $\pm$ 0.17	0.0	18.6	58.2	12.7	83.8	95.8
2c	H, H	Methyl	Phenethyl	0.53 $\pm$ 0.10	0.0	49.0	82.1	10.1	78.9	98.3
2d	–OCH ₂ CH ₂ O–	Methyl	Phenethyl	4.53 $\pm$ 0.13	0.0	35.3	41.4	7.4	66.8	95.7
2e	H, H	Methyl	2-(Morpholin-4-yl)ethyl	8.17 $\pm$ 2.22	0.0	0.0	23.9	0.0	9.6	69.6
2f	–OCH ₂ CH ₂ O–	Methyl	2-(Morpholin-4-yl)ethyl	>20	0.0	0.0	20.2	0.0	26.5	92.0
2g	H, H	Methyl	<i>iso</i> -propyl	5.69 $\pm$ 1.74	0.0	0.0	42.6	2.1	17.5	71.2
2h	–OCH ₂ CH ₂ O–	Methyl	<i>iso</i> -propyl	>20	0.0	0.0	42.2	1.7	24.6	74.9
2i	H, H	Methyl	H	0.24 $\pm$ 0.11	14.4	63.2	91.5	22.4	81.5	98.1
2j	–OCH ₂ CH ₂ O–	Methyl	H	0.81 $\pm$ 1.16	26.3	60.8	92.2	32.5	86.3	96.6
2k	H, H	Ethyl	H	0.45 $\pm$ 0.10	16.9	61.2	91.5	31.9	89.2	96.6
2l	H, H	H	H	8.73 $\pm$ 0.69	21.5	55.4	79.9	54.2	87.7	99.1
MDL 28,170				0.20 $\pm$ 0.03	96	96.6	100	100	100	100

^a Human plasma μ -calpain.

^b Bovine spleen cathepsin.

^c Human liver cathepsin.

^d Mean values of three independent experiments.

in the R_4 -position showed good inhibition of μ -calpain (IC₅₀ = 0.53–0.72 μ M), while the potencies were decreased about 10-fold when these substituents were replaced by 2-(morpholin-4-yl)ethyl or isopropyl amide. Among synthesized chromone derivatives, compound **2i**, which possesses primary amide, showed the most potent inhibitory activity (IC₅₀ = 0.24 $\pm$ 0.11 μ M) comparable to the inhibitory activity of MDL 28,170 (IC₅₀ = 0.20 $\pm$ 0.03 μ M).²² Thus, further SAR was studied on the chromone derivatives possessing primary amide by

the variation of C-3 substituent (R_3). The replacement of C-3 methyl group in **2i** with ethyl group retained potent inhibitory activity (**2k**, IC₅₀ = 0.45 $\pm$ 0.10 μ M), whereas the removal of C-3 substituent decreased the activity (**2l**, IC₅₀ = 8.73 $\pm$ 0.69 μ M).

The chromone derivatives were also evaluated for inhibition of cathepsin B and cathepsin L and their results are given in Table 1 because many of reported calpain inhibitors including MDL 28,170 (**1**) were also found

to inhibit closely related cysteine proteases owing to poor selectivity.^{9,23} Indeed, MDL 28,170 inhibited cathepsin B and cathepsin L by nearly 100% at 0.2 μ M concentration. On the other hand, chromone derivatives **2** showed moderate inhibition on both cathepsins in a dose-dependent manner. Compound **2i**, the most potent calpain inhibitor of this series, exhibited only 14.4% and 22.4% inhibition on the activities of cathepsin B and cathepsin L, respectively, at the same concentration, demonstrating high selectivity of chromone derivatives for μ -calpain.

In conclusion, chromone carboxamides as novel μ -calpain inhibitors were synthesized and evaluated for μ -calpain inhibition with a brief SAR. Among synthesized, compound **2i** derived from primary amide was the most potent calpain inhibitor with high selectivity for μ -calpain over two related cysteine proteases cathepsin B and cathepsin L. Therefore, the chromone ring can be considered as a new scaffold in designing more selective and potent μ -calpain inhibitors for treatment of calpain-associated diseases.

Acknowledgements

This research was supported by a grant (M1-0310-22-0001) from Bio-challenger Program funded by Ministry of Science and Technology, Korea.

References and notes

- Sorimachi, H.; Ishiura, S.; Suzuki, K. *Biochem. J.* **1997**, *328*, 721.
- Croall, D. E.; DeMartino, G. N. *Physiol. Rev.* **1991**, *71*, 813.
- Markgraf, C. G.; Velayo, N. L.; Johnson, M. P.; McCarty, D. R.; Medhi, S.; Koehl, J. R.; Chmielwski, P. A.; Linnik, M. D. *Stroke* **1998**, *29*, 152.
- Iwamoto, H.; Miura, T.; Okamura, T.; Shirakawa, K.; Iwatate, M.; Kawamura, S.; Tatsuno, H.; Ikeda, Y.; Matsuzaki, M. *J. Cardiovasc. Pharmacol.* **1999**, *33*, 580.
- Banik, N. L.; Shields, D. C.; Ray, S.; Davis, B.; Matzelle, D.; Wilford, G.; Hogan, E. L. *Ann. N.Y. Acad. Sci.* **1998**, *844*, 131.
- Boland, B.; Campbell, V. *Neurobiol. Aging* **2003**, *24*, 179.
- Nakamura, M.; Yamaguchi, M.; Sakai, O.; Inoue, J. *Bioorg. Med. Chem.* **2003**, *11*, 1371.
- Kunz, S.; Niederberger, E.; Ehnert, C.; Coste, O.; Pfenniger, A.; Kruij, J.; Wendrich, T. M.; Schmidtko, A.; Tegeder, I.; Geisslinger, G. *Pain* **2004**, *110*, 409.
- Mehdi, S. *Trends Biol. Sci.* **1991**, *16*, 150–153.
- (a) Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305; (b) Feherentz, J. A.; Castro, B. *Synthesis* **1983**, 676–678.
- Lubisch, W.; Hofmann, H. P.; Treiber, H. J.; Möller, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2187.
- Lubisch, W.; Möller, A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1335.
- Nakamura, M.; Inoue, J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1603.
- Bartus, R. T.; Baker, K. L.; Heiser, A. D.; Sawyer, S. D.; Dean, R. L.; Elliot, P. J.; Straub, J. A. *J. Cerebr. Blood Flow Metab.* **1994**, *14*, 537.
- Donnelly, J. A.; Maloney, D. E. *Tetrahedron* **1979**, *35*, 2883.
- Besson, T.; Nicolas Ruiz, N.; Coudert, G.; Guillaumet, G. *Tetrahedron* **1995**, *51*, 3197.
- Hauser, F. M.; Dorsch, W. A. *Org. Lett.* **2003**, *5*, 3753.
- Harbenson, S. L.; Albelleira, S. M.; Akiyama, A.; Barret, R., III; Carroll, R. M.; Straub, J. A.; Tkacz, J. N.; Wu, C. C.; Musso, G. F. *J. Med. Chem.* **1994**, *37*, 2918.
- Buroker-Kilgore, M.; Wang, K. K. W. *Anal. Biochem.* **1993**, *208*, 387.
- Hasnain, S.; Hiram, T.; Huber, C. P.; Mason, P.; Mort, J. S. *J. Biol. Chem.* **1993**, *268*, 235.
- Demuth, H. U.; Schierhorn, A.; Bryan, P.; Hofke, R.; Kirschke, H.; Bromme, D. *Biochim. Biophys. Acta* **1996**, *1295*, 179–186.
- Spectra data of selected compound **2i**: ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.41 (d, 1H, *J* = 9.0 Hz, –CO–NH–), 8.20 (s, 1H, –CO–NH₂), 8.05 (d, 1H, *J* = 7.9 Hz, aromatic), 7.93 (s, 1H, –CO–NH₂), 7.85 (t, 1H, *J* = 7.7 Hz, aromatic), 7.65 (d, 1H, *J* = 8.4 Hz, aromatic), 7.50 (t, 1H, *J* = 7.5 Hz, aromatic), 7.34–7.22 (m, 5H, aromatic), 5.47 (m, 1H, –NH–CH–CH₂–Ph), 3.29 (dd, 1H, *J* = 3.8, 13.8 Hz, –NH–CH–CH₂–Ph), 2.91 (dd, 1H, *J* = 13.8, 10.2 Hz, –NH–CH–CH₂–Ph), 1.95 (s, 3H, –C=C–CH₃); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 201.5, 183.0, 167.9, 166.5, 160.2, 158.1, 142.8, 140.1, 134.5, 133.9, 132.2, 131.2, 130.5, 127.4, 124.1, 123.9, 61.3, 40.2, 15.0; HRFABMS (positive-mode) *m/z* calcd for C₁₂H₁₉O₅N₂: 379.1294, obsd: 379.1269.
- Donkor, I. O. *Curr. Med. Chem.* **2000**, *7*, 1171.