Bioorganic & Medicinal Chemistry Letters 19 (2009) 502–507

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of 4-aryl-4-oxobutanoic acid amides as calpain inhibitors

Yong Zhang [a,b,†], Seo Yoon Jung [b], Changbae Jin [c], Nam Doo Kim [d], Ping Gong [a], Yong Sup Lee [b,*]

[a] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, Shenyang 110016, Liaoning, PR China
[b] Department of Pharmaceutical Science, College of Pharmacy & Department of Life and Nanopharmaceutical Science, Kyung Hee University, 1 Hoegi-dong Dongdaemoon-ku, Seoul 130-701, Republic of Korea
[c] Doping Control Center, Korea Institute of Science & Technology, Seoul 130-650, Republic of Korea
[d] R&D Center, Equispharm Company, Limited, Sungnam, Kyunggi-do 463-825, Republic of Korea

ARTICLE INFO

Article history:
Received 12 August 2008
Revised 3 November 2008
Accepted 11 November 2008
Available online 14 November 2008

Keywords:
Calpain inhibitor
Stroke
4-Aryl-4-oxobutanoic acid
Ketoamide
Chromone

ABSTRACT

The involvement of μ-calpain in neurological disorders, such as stroke and Alzheimer's disease has attracted considerable interest in the use of calpain inhibitors as therapeutic agents. 4-Aryl-4-oxobutanoic acid amide derivatives **4** were designed as acyclic variants of μ-calpain inhibitory chromone and quinolinone derivatives. Of the compounds synthesized, **4c-2**, which possesses a 2-methoxymethoxy group at the phenyl ring and a primary amide at the warhead region most potently inhibited μ-calpain (IC_{50} = 0.34 μM). Our findings suggest that the 4-aryl-4-oxobutanoic acid amide derivatives should be considered as a new family of μ-calpain inhibitors.

Calpains are a family of intracellular calcium-dependent cysteine proteases, which are ubiquitously expressed in many cells and tissues.[1] Two major forms of calpains have been identified: calpain I (or μ-calpain) and calpain II (or m-calpain), which are activated by micromolar and millimolar concentrations of calcium ions, respectively.[2] The calpains are required for numerous calcium-regulated cellular processes, such as signal transduction, cell proliferation, differentiation, and apoptosis. However, excessive calpain activation may cause serious cell damage or even cell death, as occurs in neurological disorders, such as stroke[3] and Alzheimer's disease.[4] Accumulated evidences of the involvement of μ-calpain in human diseases have attracted much interest in the identification of calpain inhibitors and in the elucidation of (patho)physiological roles[5,6] and their therapeutic potentials.[7]

The majority of the known calpain inhibitors bind to the catalytic site in a competitive manner, and are derived from small peptides (e.g., **1**, MDL 28,170), which are structurally related to calpain substrate cleavage sites (Fig. 1).[8,9] In connection with our on-going work to identify a new scaffold for μ-calpain inhibitors, we recently reported that chromone carboxamide **2** is a conformationally restricted cyclic analog of **1**.[10] To increase the inhibitory activity of **2**, quinolinone carboxamide **3**, which has a –NH group in place of a hydrogen bond acceptor oxygen at chromone carboxamide **2**, was designed,[11] because this group might act as a hydrogen bond donor at the active site of calpain as in **1.** However, the quinolinone carboxamide **3** showed 10-fold less potent inhibitory activity than **2** indicating that more detailed structure–activity relationship studies are required to understand the interaction between inhibitors and μ-calpain. Based on these findings, we designed 4-aryl-4-oxobutanoic acid amide derivatives **4** as alterna-

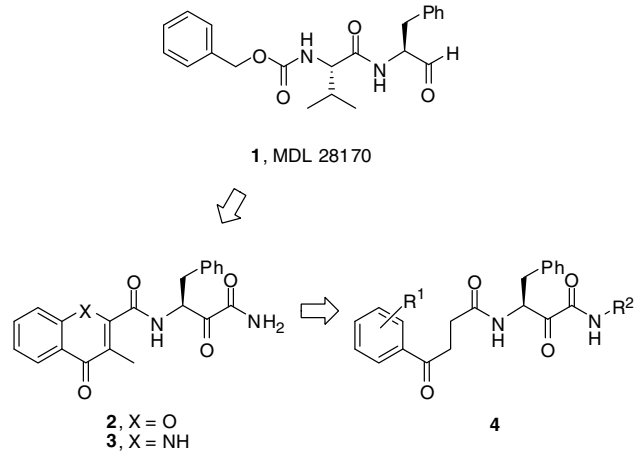


Figure 1. Schematic showing the molecular dispositions of 4-aryl-4-oxobutanoic acid amides **4**.

* Corresponding author. Tel.: +82 2 961 0370; fax: +82 2 966 2297.
E-mail address: kyslee@khu.ac.kr (Y.S. Lee).
† Present address: Hebei University of Science and Technology, Hebei, PR China.

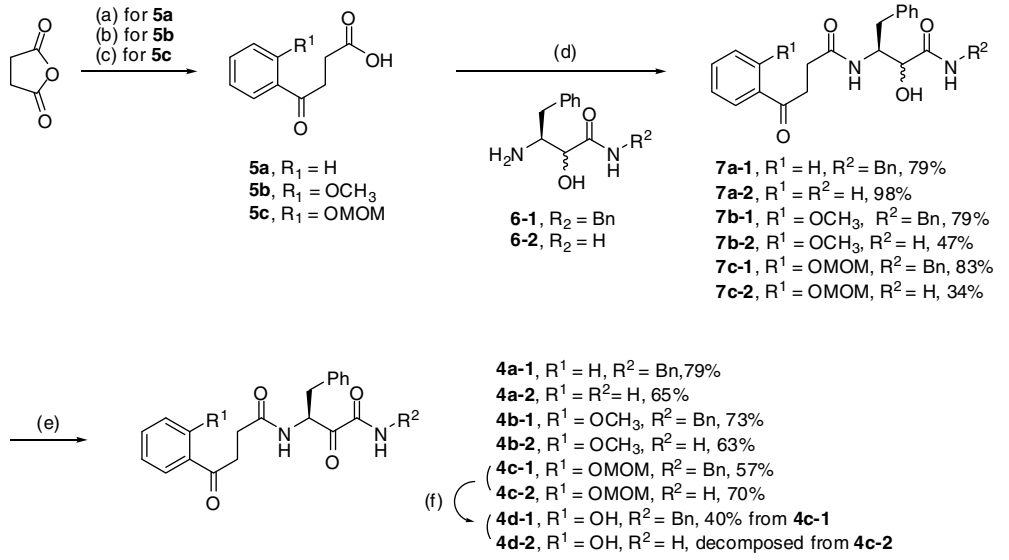
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.11.030

tive scaffold for μ-calpain inhibitors. We envisioned that the structure of **4** can be considered an acyclic analogue of **2** or **3** since it has the same functional groups. Furthermore, it was anticipated that the open chain structure of **4** might interact with μ-calpain in a similar fashion to the parent compounds, **2** and **3** due to its flexible structure. The requirement for a hydrogen bond donor or acceptor group at the active site can easily be tested by introducing methoxy or hydroxyl substituents on the aromatic ring (R^1). Herein, we describe the synthesis of 4-aryl-4-oxobutanoic acid amide derivatives **4** and their biological evaluation in terms of μ-calpain inhibition. Ketoamide was used as a warhead in the new inhibitors since several ketoamide-derived inhibitors have shown improved in vitro and in vivo metabolic stability.[12] To investigate the influence of substituents located in the warhead position of inhibitors, we also introduced variations in the amide region (R^2).

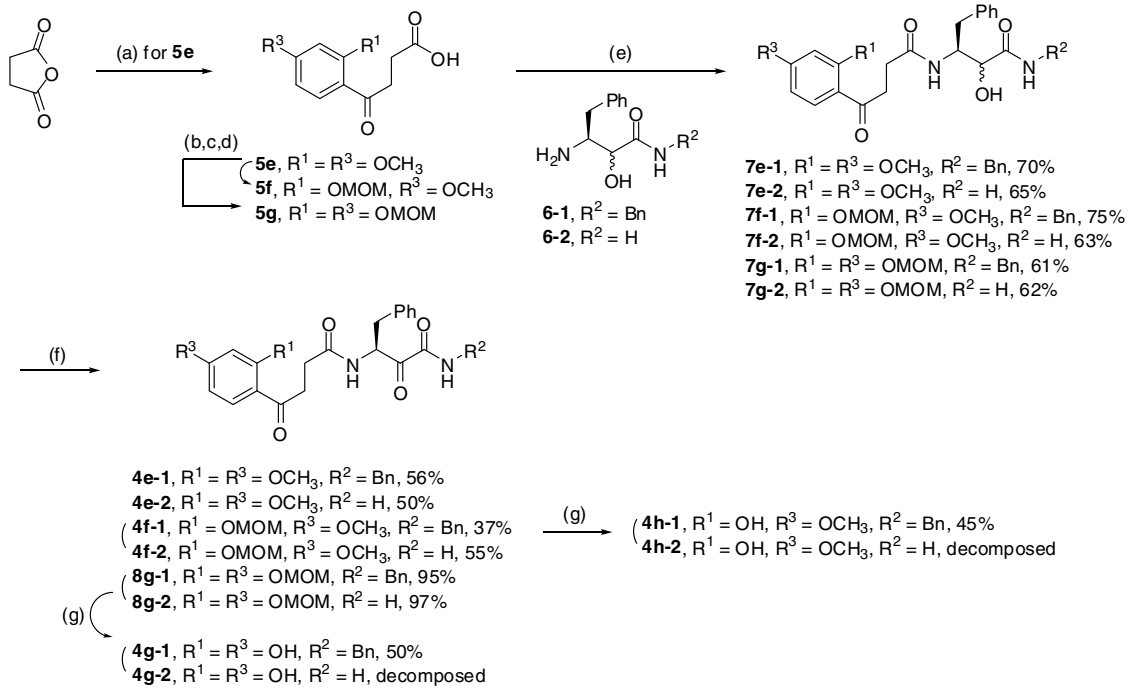
The synthesis of 4-aryl-4-oxobutanoic acid amide derivatives **4** was accomplished using a general pathway,[11] which comprises a coupling reaction of 4-aryl-4-oxobutanoic acids **5** with hydroxyl amines **6** using an EDC/HOBt system followed by oxidation of the resulting hydroxylamides **7** under Dess–Martin periodinane conditions, as illustrated in Schemes 1–3. The synthesis started with the preparation of 4-aryl-4-oxobutanoic acids via the Friedel–Crafts acylation of the appropriate benzene derivatives with succinic anhydride. For the synthesis of **4**, which contains a hydroxyl substituent on its aromatic ring, a final deprotection step was required. The synthesis of 4-aryl-4-oxobutanoic acid amide derivatives **4**, which have a phenyl or mono-substituted phenyl ring at C-4, is shown in Scheme 1. The acylation of benzene with succinic anhydride in the presence of $AlCl_3$ under reflux condition gave **5a**; to synthesize **5b** and **5c**, phenol was used in the succinoylation. The reaction between phenol and $AlCl_3$ and succinic anhydride in 1,2-dichloroethane at reflux temperature afforded 4-(2-hydroxyphenyl)-4-oxobutanoic acid,[13] and the methylation of hydroxyl and carboxyl groups in 4-(2-hydroxyphenyl)-4-oxobutanoic acid with methyl iodide and K_2CO_3 followed by hydrolysis of the resulting methyl ester with KOH in aqueous methanol afforded **5b**. Compound **5c** was prepared in three steps from 4-(2-hydroxyphenyl)-4-oxobutanoic acid via its esterification to the methyl ester, MOM-protection of the hydroxyl group, and methyl ester hydrolysis. The resulting 4-aryl-4-oxobutanoic acids **5a–c** were coupled to two hydroxylamines, **6-1** and **6-2**, which contained a benzylamide and a primary amide group, respectively, to afford the hydroxylamides **7a-1–7c-2**. These were subsequently transformed into the corresponding 4-aryl-4-oxobutanoic acid amide derivatives **4a-1–4c-2** by oxidation under Dess–Martin periodinane conditions. The compound **4d-1** was obtained by the deprotection of **4c-1** using HCl and NaI in acetone,[14] whereas compound **4d-2** could not be isolated from **4c-2**, probably due to the instability of the primary amide group under acidic deprotection conditions.

The synthesis of **4**, which has a 2,4-disubstituted phenyl ring at C-4, is shown in Scheme 2. The reaction of 1,3-dimethoxybenzene with succinic anhydride and $AlCl_3$ in 1,2-dichloroethane at 0–5 °C gave **5e**, which was transformed to **5f** and **5g** in four steps via esterification to the methyl ester using $(CH_3)_2SO_4$ and K_2CO_3 in acetone, demethylation of the methyl ethers so formed at the 2- or 4-positions with $AlCl_3$, MOM-protecting of the resulting hydroxyl groups, and hydrolysis of the methyl esters. When demethylation was carried out at room temperature, selective demethylation occurred at C-2 position of phenyl ring, whereas when conducted at reflux temperature methyl groups at the C-2 and C-4 positions were removed. The resulting 4-aryl-4-oxobutanoic acids **5e–g** were coupled to two hydroxyamines **6-1** and **6-2** to afford hydroxyamides **7e-1–7g-2**, which were transformed into the 4-aryl-4-oxobutanoic acid amide derivatives **4e-1–4f-2** and **8g-1–8g-2** by oxidation under Dess–Martin periodinane conditions. The deprotection of the MOM group in **8g-1** and **4f-1** using HCl and NaI in acetone afforded **4g-1** and **4h-1**, but compounds **4g-2** and **4h-2**, which contain a primary amide group, could not be obtained from **8g-2** and **4f-2** under deprotection conditions.

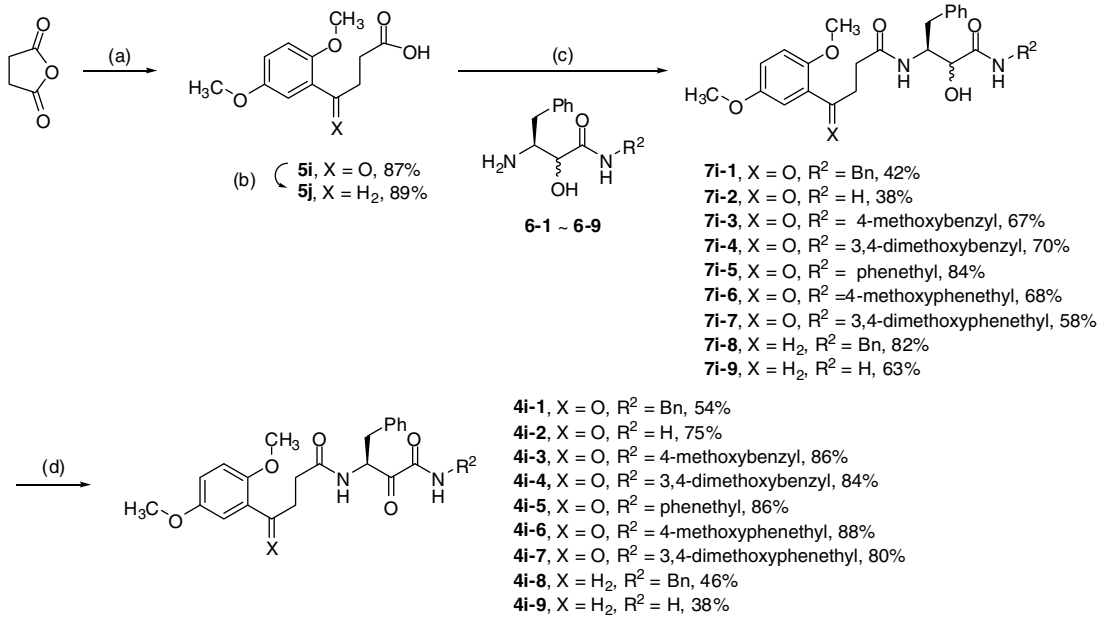
The synthesis of **4**, which has a 2,5-disubstituted phenyl ring at C-4 position is shown in Scheme 3. The reaction between 1,4-dimethoxybenzene with succinic anhydride and $AlCl_3$ in 1,2-dichloroethane at 0–5 °C gave **5i**. To examine the influence of the C-2 carbonyl group of inhibitors, compound **5i** was hydrogenated in HOAc over Pd/C at an atmosphere of hydrogen to give **5j**. The 4-aryl-4-oxobutanoic acids **5i** and **5j** were coupled to various hydroxyamines **6** and then subjected to Dess–Martin periodinane conditions to provide **4i-1–4i-9**. To examine the influences of substituents (R^2) located in the inhibitor warhead position, seven hydroxyamines (**6-1–6-9**) were used in coupling reactions. The



Scheme 1. Reagents and conditions: (a) $AlCl_3$, benzene, reflux, 4 h, 62%; (b) i—$AlCl_3$, phenol, $ClCH_2CH_2Cl$, reflux, 2 h, 19%, ii—MeI, K_2CO_3, THF, 87%, iii—KOH, MeOH, H_2O, 91%; (c) i—*p*-TsOH, MeOH, 84%, ii—MOM-Cl, NaH, DMF, 92%, iii—KOH, MeOH, H_2O, 85%; (d) EDC, HOBt, DMF; (e) Dess–Martin periodinane, CH_2Cl_2; (f) HCl, NaI, acetone, reflux.



Scheme 2. Reagents and conditions: (a) 1,3-dimethoxybenzene, $AlCl_3$, $ClCH_2CH_2Cl$, 0–5 °C, 3 days, 47%; (b) $(CH_3)_2SO_4$, K_2CO_3, acetone, 85%; (c) $AlCl_3$, CH_2Cl_2, rt for **5f** in 59% and reflux for **5g** in 92%; (d) i—MOM-Cl, NaH, DMF, ii—KOH, MeOH, H_2O, 78% for **5f** and 75% for **5g**; (e) EDC, HOBt, DMF; (f) Dess–Martin periodinane, CH_2Cl_2; (g) HCl, NaI, acetone, reflux.



Scheme 3. Reagents and conditions: (a) 1,4-dimethoxybenzene, $AlCl_3$, $ClCH_2CH_2Cl$, 0–5 °C, 3 days, 87%; (b) H_2, 10% Pd/C, AcOH; (c) EDC, HOBt, DMF; (d) Dess–Martin periodinane, CH_2Cl_2.

yields of these coupling and oxidation reactions are summarized in Scheme 3.[15]

The μ-calpain inhibitory activities of the prepared 4-aryl-4-oxobutanoic acid amide derivatives **4** were evaluated using human calpain I isolated from erythrocytes. Suc-Leu-Tyr-AMC was used as the fluorogenic substrate.[11] MDL 28,170 (**1**), chromone derivative **2**,[10] and the quinolinone derivative **3**[11] were also tested and compared and assay results are summarized in Tables 1 and 2. First, we investigated the influence of substituents at the phenyl ring (R^1 and R^3) of compounds on μ-calpain activity. The amide groups in the warhead position (R^2) were tentatively fixed as benzyl and primary amides. When substituents were incorporated at the 2-position of the C-4 phenyl ring, a clear structure–activity relationship was found, as summarized in Table 1. Methoxy- or methoxymethoxy-substituted derivatives (entries 2–3, 5–6, and 8) showed more potent inhibitory activities than unsubstituted derivatives (entry 1). Of the compounds synthesized, **4c-2** most potently inhibited μ-calpain activity with an IC_{50} value of 0.34 μM, which

Table 1
The μ-calpain inhibitory activities of 4-aryl-4-oxobutanoic acid amides **4a-1**–**4i-2**

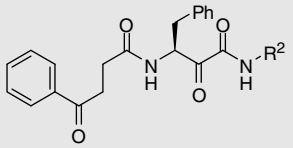
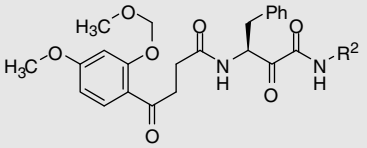
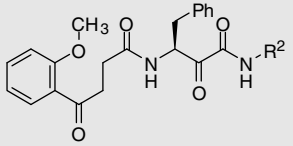
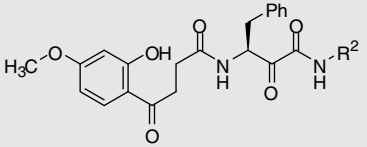
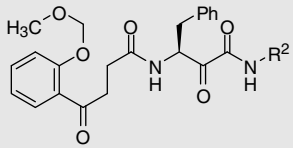
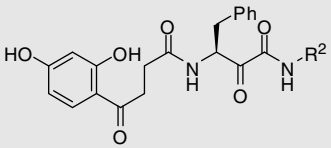
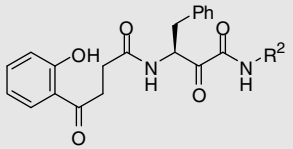
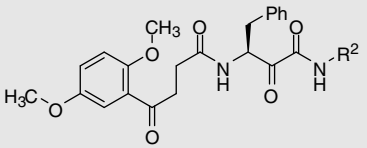
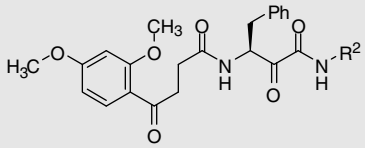
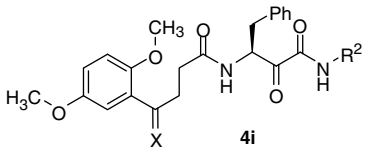
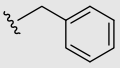
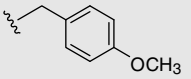
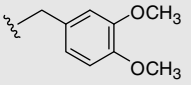
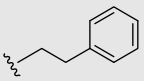
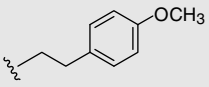
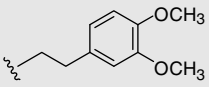
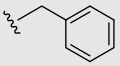
Entry	Structures	Compounds	Calpain inhibition IC_{50} (μM)	Entry	Structures	Compounds	Calpain inhibition IC_{50} (μM)
1		**4a-1**, R^2 = Bn **4a-2**, R^2 = H	6.25 ± 0.10 3.20 ± 0.06	6		**4f-1**, R^2 = Bn **4f-2**, R^2 = H	4.25 ± 0.51 0.70 ± 0.03
2		**4b-1**, R^2 = Bn **4b-2**, R^2 = H	3.33 ± 0.19 0.54 ± 0.02	7		**4g-1**, R^2 = Bn **4g-2**, R^2 = H, dec	12.99 ± 0.94 —
3		**4c-1**, R^2 = Bn **4c-2**, R^2 = H	4.29 ± 0.81 0.34 ± 0.02	8		**4h-1**, R^2 = Bn **4h-2**, R^2 = H, dec	5.73 ± 0.20 —
4		**4d-1**, R^2 = Bn **4d-2**, R^2 = H, dec	13.84 ± 1.91 —	9		**4i-1**, R^2 = Bn **4i-2**, R^2 = H	3.77 ± 0.43 0.52 ± 0.01
5		**4e-1**, R^2 = Bn **4e-2**, R^2 = H	6.16 ± 0.45 1.18 ± 0.09	10 11 12	MDL 28170, 1 Chromone carboxamide 2 Quinolinone carboxamide 3		0.07 ± 0.01 0.04 ± 0.01 0.71 ± 0.07

Table 2
The μ-calpain inhibitory activities of 4-aryl-4-oxobutanoic acid amides **4i-1**–**4i-9**



4i

Compound	X	R^2	Calpain inhibition IC_{50} (μM)
4i-1	X = O		3.77 ± 0.43
4i-2	X = O	H	0 52 ± 0.01
4i-3	X = O		2.35 ± 0.13
4i-4	X = O		1.64 ± 0.07
4i-5	X = O		2.09 ± 0.05
4i-6	X = O		0.95 ± 0.02
4i-7	X = O		2.13 ± 0.17
4i-8	$X = H_2$		8.00 ± 1.44
4i-9	$X = H_2$	H	1.86 ± 0.03

was approximately 8-fold less potent than that of the parent chromone derivative **2**, but 2-fold more potent than that of quinolinone **3** to reveal that open chain structures of bicyclic chromones or quinolinones can also bind well at the active site of μ-calpain. On the other hand, substitution of the hydroxyl group at the 2-position of the C-4 phenyl ring reduced inhibitory activities (entries 4 and 7) indicating that the hydrogen bond donor group at the C-4 phenyl ring is more efficient at binding the active site of calpain than the hydrogen bond acceptor –OH in this series of compounds.

Regarding amide groups in the warhead position (R^2), compounds derived from small primary amides were always more potent inhibitors than those derived from benzyl amide. However, primary amide-derived compounds **4d-2**, **4g-2**, and **4h-2**, which have a hydroxyl group at the 2-position of the C-4 phenyl ring were not obtained probably because of their instabilities during removal of the MOM-protecting group under acidic conditions. Therefore, we tried to replace the unstable primary amide group with *N*-alkylaryl amide groups in the warhead region; the inhibitory activities of the resulting compounds are summarized in Table 2. The 4-(2,5-dimethoxyphenyl)-4-oxobutanoic acid was chosen for this study because of the ready availability by simple Friedel–Crafts reaction of 1,4-dimethoxybenzene with succinic anhydride and the potent inhibitory activity of its derivative **4i-2**. In terms of the benzyl amide derivatives, it was observed that the inhibitory activity increased as the number of methoxy substituents increased. Of the *N*-alkylaryl amide derivatives synthesized, the 4-methoxyphenethyl amide **4i-6** was most potent with an IC_{50} value of 0.95 μM, although its activity was slightly lesser than that of the primary amide derivative **4i-2** (IC_{50} = 0.52 μM). These findings suggest that the 4-methoxyphenethyl amide group can be used in place of a primary amide in the warhead position without causing substantial activity loss when the synthesis of primary amide-derived μ-calpain inhibitors is difficult. The removal of the C-2 carbonyl oxygen of inhibitors (**4i-8**, **4i-9**) was found to reduce inhibition, indicating that the oxygen group is required in this series of compounds for μ-calpain inhibition.

In conclusion, 4-aryl-4-oxobutanoic acid amide derivatives **4** were synthesized as acyclic structural variants of the μ-calpain inhibitory chromone and quinolinone derivatives in order to elucidate the structural requirements for inhibitor binding to the active site of μ-calpain. The hydrogen bond acceptor groups, like the methoxy-substituents were more efficient at binding at the active site of μ-calpain than hydrogen bond donor groups. Of the compounds synthesized, **4c-2**, which possesses a 2-methoxymethoxy group in the phenyl ring and a primary amide in the warhead region, most potently inhibited μ-calpain (IC_{50} = 0.34 μM). These findings indicate the 4-aryl-4-oxobutanoic acid amide derivatives should be considered a new family of μ-calpain inhibitors. Furthermore, the study also shows that a 4-methoxyphenethyl amide group can be used to replace a chemically labile primary amide in the inhibitor's warhead position.

Acknowledgments

This research was supported by the Mid-Term Technological Development Project funded by the Korean Ministry of Commerce, Industry and Energy (Grant No. 10027898-2007-22) and by the Seoul Research and Business Development Program (10524).

References and notes

1. Suzuki, K.; Sorimachi, H.; Yoshizawa, T.; Kinbara, K.; Ishiura, S. *Biol. Chem.* **1995**, *376*, 523.
2. Croall, D. E.; DeMartino, G. N. *Physiol. Rev.* **1991**, *71*, 813.
3. Markgraf, C. G.; Velayo, N. L.; Johnson, M. P.; McCarty, D. R.; Medhi, S.; Koehl, J. R.; Chmielwski, P. A.; Linnik, M. *Stroke* **1998**, *29*, 152.
4. Boland, B.; Campbell, V. *Neurobiol. Aging* **2003**, *24*, 179.
5. Sha, D.; Jin, Y.; Wu, H.; Wei, J.; Lin, C.-H.; Lee, Y.-H.; Buddhala, C.; Kuchay, S.; Chishti, A. H.; Wu, J.-Y. *Brain Res.* **2008**, *1207*, 9.
6. Kuchay, S. M.; Chishti, A. H. *Curr. Opin. Hematol.* **2007**, *14*, 249.
7. Neffe, A. T.; Abell, A. D. *Curr. Opin. Drug Discov. Dev.* **2005**, *8*, 684.
8. Mehdi, S. *Trends Biochem. Sci.* **1991**, *16*, 150.
9. Shirasaki, Y.; Yamaguchi, M.; Miyashita, H. *J. Ocul. Pharmacol. Ther.* **2007**, *22*, 417.
10. Lee, K. S.; Seo, S. H.; Lee, Y. H.; Kim, H. D.; Son, M. H.; Chung, B. Y.; Lee, J. Y.; Jin, C.; Lee, Y. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2857.
11. Nam, D. H.; Lee, K. S.; Kim, S. H.; Kim, S. M.; Jung, S. Y.; Chung, S. H.; Kim, H. J.; Kim, N. D.; Jin, C.; Lee, Y. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 205.
12. Lubisch, W.; Beckenbach, E.; Bopp, S.; Hofmann, H.-P.; Kartal, A.; Kaestel, C.; Lindner, T.; Metz-Garrecht, M.; Reeb, J.; Regner, F.; Vierling, M.; Moeller, A. *J. Med. Chem.* **2003**, *46*, 2404.
13. Fieser, L. F.; Gates, M. D.; Kilmer, G. W. *J. Am. Chem. Soc.* **1940**, *62*, 2966.
14. Huffman, J. W.; Zhang, X.; Wu, M.-J.; Joyner, H. H. *J. Org. Chem.* **1991**, *56*, 1481.
15. Spectral data of selected compounds. Compound **4c-2**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (d, J = 7.2 Hz, 1H, –*NH*), 8.00 (s, 1H, –*NH*), 7.76 (s, 1H, –*NH*), 6.95–7.54 (m, 9H, *aromatic*), 5.29 (s, 2H, –O*CH*$_2$O–), 5.19 (m, 1H, –NH–*CH*–CH$_2$–Ph), 3.39 (s, 3H, –O*CH*$_3$), 3.07–3.12 (m, 2H, –*CH*$_2$–), 2.74 (dd, J = 14.0, 9.6 Hz, 1H, –CH–*CH*$_2$Ph), 2.43–2.49 (m, 3H, –*CH*$_2$–, –CH–*CH*$_2$Ph); ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.9, 197.4, 172.0, 163.2, 155.8, 138.0, 133.7, 130.0, 129.5 (2C), 129.1, 128.7 (2C), 126.9, 121.9, 115.6, 94.6, 56.6, 55.5, 38.9, 35.7, 29.6. Compound **4i-2**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, J = 7.2 Hz, 1H, –*NH*), 8.02 (s, 1H, –*NH*), 7.78 (s, 1H, –*NH*), 7.07–7.31 (m, 8H, *aromatic*), 5.19 (m, 1H, –NH–*CH*–CH$_2$–Ph), 3.82 (s, 3H, –O*CH*$_3$), 3.73 (s, 3H, –O*CH*$_3$), 3.02–3.12 (m, 3H, –*CH*$_2$–, –CH–*CH*$_2$Ph), 2.74 (dd, J = 14.0, 9.6 Hz, 1H, –CH–*CH*$_2$Ph), 2.41–2.45 (m, 2H, –*CH*$_2$–); ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.2, 197.5, 172.0, 163.2, 153.3, 153.0, 138.1, 129.5 (2C), 128.7 (2C), 128.3, 126.9, 119.8, 114.5, 114.1, 56.7, 56.0, 55.5, 39.0, 35.7, 29.6. Compound **4i-6**: ^{1}H NMR (400 MHz,

DMSO-d_6) δ 8.71 (t, J = 6.0 Hz, 1H, –*NH*), 8.33 (d, J = 7.2 Hz, 1H, –*NH*), 7.05–7.31 (m, 10H, *aromatic*), 6.85 (d, J = 8.4 Hz, 2H, *aromatic*), 5.17 (m, 1H, –NH–*CH*–CH_2–Ph), 3.80 (*s*, 3H, –OCH_3), 3.72 (s, 3H, –OCH_3), 3.67 (s, 3H, –OCH_3), 3.26–3.38 (m, 2H, –CH_2–), 3.00–3.07 (m, 3H, –CH–CH_2Ph, –CH_2–), 2.56–2.73 (m, 3H, –CH–CH_2Ph, –CH_2–), 2.41–2.45 (m, 2H, –CH_2–); ^{13}C NMR (100 MHz, DMSO-d_6) δ 199.6, 196.5, 171.5, 160.6, 157.7, 152.8, 152.5, 137.5, 130.8, 129.5 (2C), 129.0 (2C), 128.2 (2C), 127.8, 126.4, 119.3, 114.0, 113.7 (2C), 113.6, 56.2, 55.5, 55.3, 54.9, 40.3, 38.5, 35.1, 33.6, 29.1.