

Development of Novel Small Chemical Inhibitors for Lck SH Domain with *in vitro* T-cell Inhibitory Activity

See-Hyoung Park,^a Mi-Ae Kang,^b Hyeong-Soo Shim, Hyeongjin Cho, Jonghwa Won,[†] and Keun-Hyeung Lee*

Department of Chemistry, Inha University, Incheon 402-751, Korea. *E-mail: leekh@inha.ac.kr

[†]Signal Transduction Laboratory, Mogam Biotechnology Research Institute, Yongin, Kyunggi-Do 449-910, Korea

Received July 7, 2006

We investigated *in vitro* T-cell inhibitory activity and bioavailability of small chemical inhibitors for Lck SH2 domain, which had a different scaffold such as an amide bond, reduced amide bond, N-methyl amide bond, thioamide bond, and urethane bond. Each of these compounds, with its particular scaffold, showed a different logP value, stability against serum enzyme, stability in buffer solution, and *in vitro* T-cell inhibitory activity. Overall results indicated that the SH2 inhibitor containing urethane bond can be a new lead compound because of its superior bioavailability, potent *in vitro* T-cell inhibitory activity, and facile synthesis.

Key Words : T-cell inhibition, Bioavailability, Antagonist, Scaffold, Lck SH2

Introduction

Src homology 2 (SH2) domains play a critical role in organizing coherent signal transducing complexes that are essential for the appropriate cellular response to extracellular stimuli.¹ SH2 domains mediate specific protein-protein interactions through binding to specific, phosphotyrosine (pY)-containing peptide sequences in their targets.² The blockade of SH2 domain-dependent protein-protein interactions has emerged as a new strategy for treating a plethora of diseases related to cell signaling, such as cancer, osteoporosis, allergy, asthma, and inflammation.³ In addition to their therapeutic potentials, SH2 antagonist (inhibitors) are regarded as useful probes for elucidating the cell signal pathways mediated by SH2 domains. Thus, inhibitors have been developed against a variety of SH2 domains, such as Src, Lck, ZAP-70, and Grb2.

Since the natural ligands for various SH2 domains are pY containing proteins, most inhibitors for SH2 domains contain pY isosteres and amide bonds.^{1,2} The negative charges of the pY isostere of the inhibitors are required to interact with the highly positively charged binding pocket of the SH2 proteins, however these negative charges decrease the cell penetrating ability of the inhibitors, resulting in a decrease in their *in vitro* cell inhibition activity. Even though non-peptide inhibitors for various SH2 domains were successfully synthesized and found to exhibit moderate binding affinity for SH2 proteins, most did not show *in vitro* cell inhibitory activity, due to their low cell membrane permeability and/or low stability against enzymes.^{3,4} As an alternative approach, we identified a small chemical inhibitor, rosmarinic acid (RosA), by screening natural products for inhibiting interactions between the Lck SH2 domain and phosphopeptide ligands.⁵ As shown in Table 1, RosA is a

relatively low charged chemical compared to the recently developed non-peptide inhibitors for SH2 domains. The small chemical inhibitor, RosA, was found to inhibit TCR-induced T cell activation and proliferation in an Lck dependent manner.^{5,6} However, RosA was not easily isolated from natural sources on the gram scale and the total chemical synthesis of the compound required several tedious steps each with a low yield.^{7,8} Furthermore, RosA was reported to be hydrolyzed readily *in vivo* because of its labile ester bond.⁹ Thus, we designed and synthesized various derivatives of RosA, which have different scaffolds.¹⁰ Even though the derivatives of RosA showed *in vitro* binding affinity to SH2 domain, the *in vitro* T-cell inhibitory activity and bioavailability of the analogs were not investigated.

In the present study, we synthesized new analogs of RosA containing urethane bond, and investigated the bioavailability and *in vitro* T-cell inhibitory activity of the SH2 inhibitors containing different scaffolds such as amide bond, reduced amide bond, N-methyl amide bond, thioamide bond, and urethane bond. Each of these compounds, with its particular scaffold, showed a different logP value, stability against serum enzyme, stability in buffer solution, and *in vitro* T-cell inhibitory activity. Some SH2 inhibitor employed in this study showed a more potent *in vitro* T-cell inhibitory activity of RosA, the previous lead compound. Unexpectedly, the analog containing thioamide bond has the shortest half life among the analogs. The overall results revealed that these small chemical inhibitors containing a urethane bond may be novel lead compounds for Lck SH2 inhibitors, because of their facile synthesis, potent *in vitro* T-cell inhibition activity, and good bioavailability.

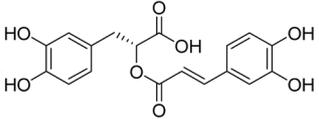
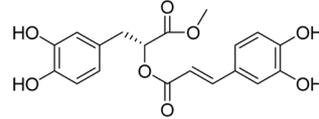
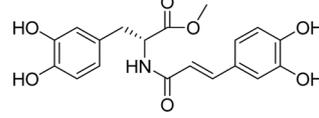
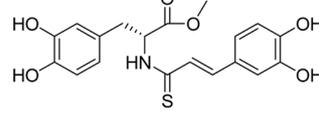
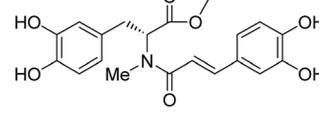
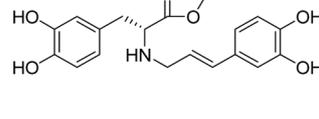
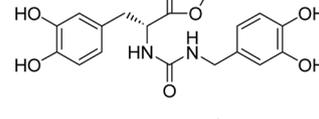
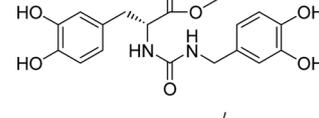
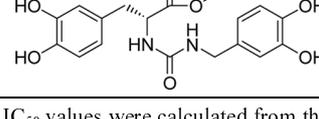
Results and Discussion

Chemistry. Compounds 6-8 were synthesized as shown in Scheme 1. The treatment of compound 9 with the corresponding alcohol in the presence of SOCl₂ as an activating agent provided ester compound 10 in considerable yield.

^aCurrent address; Department of Comparative Pathology, One Shields Avenue, University of California, Davis, Davis, CA 95616, USA

^bCurrent address; Department of Immunology, One Shields Avenue, University of California, Davis, Davis, CA 95616, USA

Table 1. Chemical properties, Lck SH2 binding affinity, and T-cell inhibitory activity of the compounds

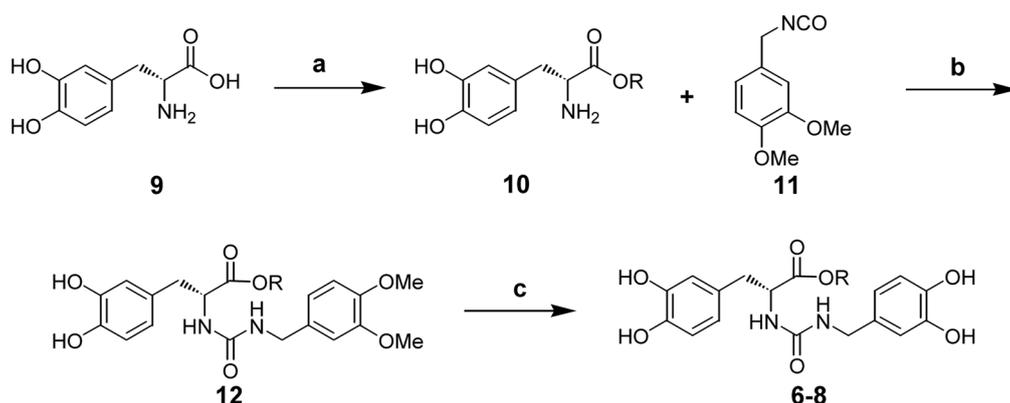
No.	Structure	Lck SH2 binding activity ^a	T-cell inhibitory activity ^b	Half-Life in serum ^c	Log P	CH ₃ CN (%)
		IC ₅₀ (μM)	% Inhibition (10 μM)	min		
RosA		24 ^d	40.4	37.2	0.834	28
1		80 ^d	54.7	38.5	1.479	30
2		121 ^d	82.6	23.1	1.386	21
3		162 ^d	59.3	>500	1.458	30
4		149 ^d	37.5	>500	1.402	23
5		>500 ^d	0	ND	-0.557	17
6		98	35.5	>500	1.177	19
7		111	49.1	>500	1.197	22
8		147	58.7	>500	1.460	28

^aThe average IC₅₀ values were calculated from three independent experiments performed in duplicate, which provided a standard deviation below 20% and the IC₅₀ value of AcPYEEIE was 1.8 μM. ^bCyclosporin A as a positive control inhibited T-cell activation completely at 10 μM, whereas RosA inhibited T-cell activation by about 40% at 10 μM. ^cThe kinetic analysis of the degradation of the peptides in the presence of serum was carried out by a linear least square analysis of the logarithm of the peak area versus time. Each half-life was determined from three independent experiments performed in duplicate. ^dThe IC₅₀ values were taken from [10].

Compound **11** was obtained from 3,4-dimethoxyphenyl azide by Curtius rearrangement. The reaction of the isocyanate **11** with the corresponding amine **10**, followed by the hydrolysis of the dimethylether of **12** by BBr₃ provided compounds **6-8** in a reasonable yield. The synthesized products were further purified by preparative reverse phase C₁₈ HPLC. The high purity (>95%) of the products was

confirmed by RP C₁₈ analytical HPLC and the success of the synthesis was confirmed by NMR and ESI-mass spectrometry.

Biological activity. The binding affinity of the compounds for the Lck SH2 domain was measured by using a competitive assay as described.¹¹ A control phosphopeptide, AcPYEEIE, exhibited approximately 1.8 μM of IC₅₀ value,



Scheme 1. Synthesis of compounds **6-8**. Reagents: (a) ROH, R = -CH₃, -CH₂CH₃, -CH(CH₃)₂; SOCl₂, 0 °C, 4 hr; (b) DCM/DMF, TEA, r.t. for 13 hr, (c) BBr₃ in DCM 1.0 M solution, DCM, 0 °C → r.t. for 18 hr.

which is highly consistent with the previously reported value¹¹ and the measured IC₅₀ values of each compound were summarized in Table 1. *In vitro* T-cell inhibitory activity of the compounds was measured by evaluating their ability to block TCR-induced IL-2 gene activation, as previously described.¹² The commercially available immune suppressive drug, cyclosporin A (CsA), was used as a positive control in this assay. As shown in Figure 1, CsA inhibited T-cell activation by almost 100% at a concentration of 10 μM, whereas RosA inhibited T-cell activation by about 40% at the same concentration. Thus, we compared the *in vitro* T-cell inhibitory activity of the compounds with the different scaffolds at a concentration of 10 μM, as shown in Table 1. The logP value and retention time of each compound are also summarized in Table 1. The retention time on the C₁₈ reverse phase HPLC column using CH₃CN-H₂O as the eluent was regarded as a good parameter for reflecting the hydrophobic interactions between the compound and the C₁₈ stationary phase.¹³

Compound **1**, the methylester form of RosA, showed more potent *in vitro* T-cell inhibitory activity than RosA, which can be explained by the fact that the former (logP, 1.479) has higher hydrophobicity than RosA (logP, 0.834). Compounds **1-4** exhibited similar or improved *in vitro* T-cell inhibitory activity compared to that of RosA. Compound **5** showed no T-cell inhibitory activity due to its low cell penetrating ability (logP, -0.557) and low binding affinity for Lck SH2 protein. As shown in Table 1, compound **2** showed the most potent T-cell inhibitory activity and compound **3** and **8** had a considerable T-cell inhibition activity. The comparison between their *in vitro* T-cell inhibitory activities and Lck SH2 protein binding activities indicated that *in vitro* SH2 domain binding affinity as well as the hydrophobicity of the inhibitors was likely to play a critical role in their *in vitro* T-cell inhibitory activity.

In vivo chemical stability can be gauged by determining the *in vitro* stability in serum.¹⁴ Thus, we measured the stability of the compounds in the presence of serum, as previously described.¹⁵ As shown in Table 1, compounds **1** and **2** which had an ester bond and amide bond, respectively,

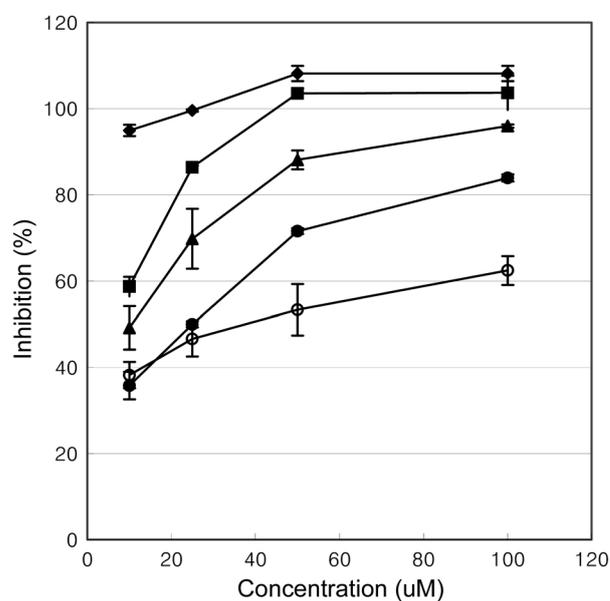


Figure 1. *In vitro* T-cell inhibitory activities of the compounds. IL-2 promoter assays were performed in duplicate and representatives of three independent experiments are shown. Cyclosporin A and rosmarinic acid were used as a positive control. ◆, cyclosporin A; ○, rosmarinic acid; ●, compound **6**; ▲, compound **7**; ■, compound **8**.

did not show good resistance against serum enzymes; the half life of compound **2** was the shortest in the series ($t_{1/2}$ = 23.1 min). Compounds **3**, **4**, and **6**, which contained an N-methyl amide bond, thioamide bond and urethane bond, respectively, showed good resistance against serum enzymes; no considerable degradation was observed for 500 min.

We also investigated the stability of the compounds in phosphate buffer solution (pH 7.4). No degradation was observed during the incubation of compounds **3**, **4**, **6**, **7**, and **8** for 3 days, which supported that they had a good stability in this buffer solution (data not shown). However, unexpectedly, compound **3** containing thioamide bond degraded in the buffer solution and its half life was much shorter than that of RosA, as shown in Figure 2. Generally, thioamide

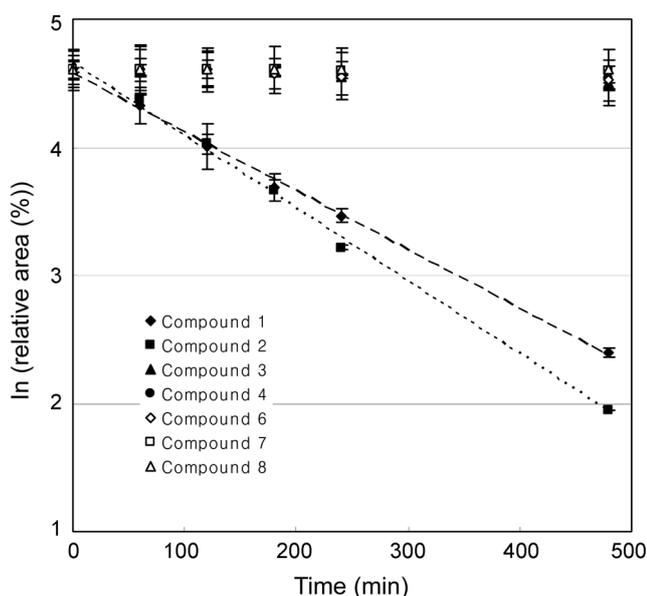


Figure 2. The stability of the compounds in the presence of serum. Each compound ($100 \mu\text{g/mL}$) was incubated in the presence of serum at 37°C and then a sample of the solution ($200 \mu\text{L}$) was removed at known times and analyzed by reverse-phase HPLC. Each half-life was determined from three independent experiments performed in duplicate.

bond is regarded as a possible isostere for amide bond due to its greater stability against enzymes.¹⁶ However according to our result, even though thioamide bond had a great resistance against serum enzymes, it was not a proper isostere for the ester bond of RosA due to its low stability in buffer solution. The log P value, stability, binding affinity for SH2 domain, and T-cell inhibitory activity of the compounds were compared in Table 1. Compound 2 showed the most potent inhibitory activity but had a shortest half life in the presence of serum enzyme. Compounds 7 and 8 containing urethane bond had considerable T-cell inhibition activity, higher logP values, and a great resistance against serum enzymes ($t_{1/2} > 8$ hrs) and in pH 7.4 buffer solution. As shown in Figure 1, compound 8 inhibited T-cell activation completely at a concentration of $50 \mu\text{M}$, whereas rosmarinic acid did not afford complete inhibition even at a concentration of $100 \mu\text{M}$. The great stability against serum enzyme and in buffer solution, facile synthesis, and the potent *in vitro* T-cell inhibitory activity indicated that compounds 7 and 8 containing urethane bond might be new lead compounds in the development of SH2 inhibitor for T-cell inhibitory agents.

In summary, we evaluated binding affinity for Lck SH2 domain, stability against enzyme and in buffer solution, log P, and T-cell inhibitory activity of the analogs of rosmarinic acid, which had a different scaffold. On the basis of these results, we successfully synthesized the novel lead compound for SH2 inhibitor that had both superior bioavailability and *in vitro* T-cell inhibitory activity. The potent *in vitro* T-cell inhibitory activity, stability against serum enzymes and facile synthesis of the compounds containing urethane bond

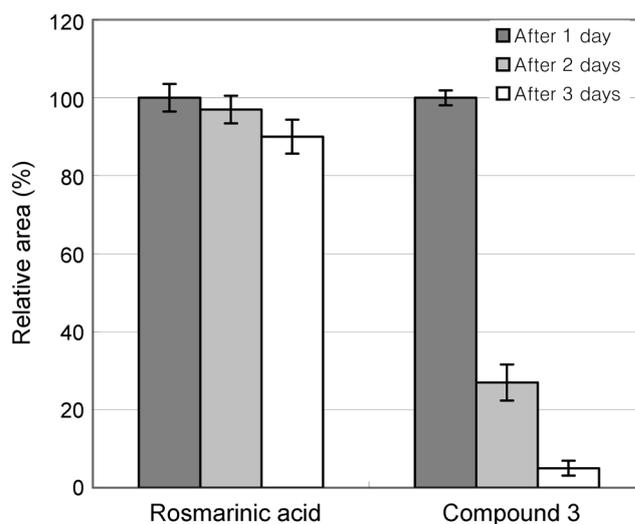


Figure 3. The stability of the compounds in buffer solution. Sample solution ($100 \mu\text{g/mL}$) was incubated at 37°C buffer solution (pH 7.4). The initial time was recorded and $100 \mu\text{g/mL}$ of the solution was removed at known time intervals and was analyzed by reverse-phase HPLC.

suggest that it could act as a promising new lead compound in the search for an effective Lck SH2 antagonist with T-cell inhibitory agent.

Experimental Section

Materials. SH2-GST fusion Lck (120-226) and polyclonal rabbit anti-GST antibody were purchased from Santa Cruz Biotechnology. (Santa Cruz, CA, USA) Horseradish peroxidase-conjugated mouse anti-rabbit antibody, peroxidase substrate (1-step Turbo TMB-ELISA, trimethylbenzidine), and streptavidin coated 96-well plates were purchased from Pierce. (Rockford, IL, USA). RosA was obtained from Indofine Chemical Company (Somerville, NJ, USA). CyclosporinA was kindly provided by Dr. G. Lee (Hanmi Pharm. Co., Korea). D-3,4-dihydroxyphenylalanine (D-DOPA) was obtained from Sigma (St. Louis, USA) All chemicals were reagent grade and used without further purification.

General procedure for the synthesis of the compounds containing urethane bond. To a solution of D-3,4-dihydroxyphenylalanine (1.2 g, 6.086 mmole) in ethanol (5 mL) was dropwise added thionylchloride (2.64 mL, 30.43 mmol) under an ice-cooled condition and stirred for 10 h. After concentration, the residue was dissolved in EtOAc and washed with dilute HCl to give object compound, DOPA ethylester (809 mg, 59%). To a solution of dimethoxyphenylisocyanate (1 g, 5.18 mmol) in 20 mL CH_2Cl_2 , were dropwise added DOPA ethylester (1.46 g, 6.21 mmol) in DMF (2 mL) and Et_3N (2.16 mL, 15.53 mmol) and stirred for 18 h. After concentration, the residue was dissolved in EtOAc. The solution was washed with dilute HCl and brine and dried over anhydrous Na_2SO_4 . A crude residue was purified by silica gel column chromatography (*n*-hexan: EtOAc : MeOH = 4 : 5 : 1) to provide the object compound 7

(1.26 g, 60.2%). Synthesis of compounds **6** and **8** were accomplished by coupling isocyanate **11** with the corresponding DOPA ester in DMF in fashions similar to that described for the synthesis of **7**.

2-[3-(3,4-Dihydroxy-benzyl)-ureido]-3-(3,4-dihydroxy-phenyl)-propionic acid methylester, Compound 6 (65% yield), $^1\text{H NMR}$ (200 MHz, DMSO) δ 6.67-6.036 (m, 6H), 4.28 (q, $J = 16$ Hz, 1H), 4.04 (s, 2H), 3.59 (s, 3H), 2.81-2.67 (m, 2H); ESI MS ($M + H^+$); Calcd. 374.14, Obs. 374.91.

2-[3-(3,4-Dihydroxy-benzyl)-ureido]-3-(3,4-dihydroxy-phenyl)-propionic acid ethylester, Compound 7 (60.2% yield), $^1\text{H NMR}$ (200 MHz, DMSO) δ 6.85-6.38 (m, 6H), 4.34 (q, $J = 40$ Hz, 1H), 4.03 (s, 2H), 4.01 (q, $J = 27.2$ Hz, 2H), 2.94-2.77 (m, 2H), 1.18 (t, $J = 12.8$ Hz, 3H); ESI MS ($M + H^+$); Calcd. 391.14, Obs. 371.92.

2-[3-(3,4-Dihydroxy-benzyl)-ureido]-3-(3,4-dihydroxy-phenyl)-propionic acid isopropylester, Compound 8 (40% yield), $^1\text{H NMR}$ (200 MHz, DMSO) δ 6.64-6.38 (m, 6H), 4.83 (m, 1H), 4.26 (q, $J = 12.4$ Hz, 1H), 3.98 (s, 2H), 2.73-2.67 (m, 2H), 1.15 (d, $J = 6$ Hz, 3H), 1.09 (d, $J = 6$ Hz, 3H); ESI MS ($M + H^+$); Calcd. 405.16, Obs. 405.78.

ELISA binding assay. The assays were performed as we described previously.^{20,24} Briefly, an enzyme-linked immunosorbant assay was employed to measure the binding affinity of synthesized derivatives for each SH2 domains. 100 μL of biotiny- ϵ -aminocaproyl-EPQpYEEIPIYL (10 ng/mL in 50 mM Tris, 150 mM NaCl pH 7.5) was added to each well of streptavidin-coated 96-well micro titer plates. A 50- μL solution of samples (1 mM, in BSA-T-TBS) and a 50- μL solution of the SH2-GST fusion proteins (100 ng/mL, in BSA-T-TBS) were added in each well and the plate was shaken for 1 hr at room temperature. The solutions were removed and each well rinsed with $4 \times 200 \mu\text{L}$ BSA-T-TBS. 100 μL of polyclonal rabbit anti-GST antibody (1 $\mu\text{g}/\text{mL}$ in BSA-T-TBS) was then added to each well and incubated for 1 hr at room temperature. After a series of final wash steps ($4 \times 200 \mu\text{L}$ BSA-T-TBS; $2 \times 300 \mu\text{L}$ TBS), 100 μL of peroxidase substrate (1-Step Turbo TMB-ELISA, trimethylbenzidine) was added to each well and incubated for 5-15 min. 100 μL of 1 M sulfuric acid solution was introduced to stop the peroxidase reaction and absorbance was measured at 450 nm with a plate reader.

Luciferase assay. The assays were performed as we described previously.²⁰ Briefly, a total of 2×10^6 Jurkat T cells were transfected with 2 μg of IL-2-Luciferase (gifts of Gerald Crabtree, Stanford University, Stanford, CA) using Lipofectamine according to manufacturer's protocol (Gibco-BRL). After incubation with DNA-Lipofectamine mixtures for 24 hours, cells were pre-incubated in the presence or absence of rosmarinic acid or its derivative (10 $\mu\text{g}/\text{mL}$) for 2 hours before stimulation. Cells were activated either with anti-CD3 mAb (5 $\mu\text{g}/\text{mL}$, UCHT1, IgG_{2a} isotype) coated on the plate or with PMA (5 $\mu\text{g}/\text{mL}$) and ionomycin (0.5 $\mu\text{g}/\text{mL}$) for 16 hours. In the rosmarinic acid or its derivative treated group, they were present through the whole 16 hours incubation process. After stimulation, the cells were washed, lysed, and assayed for luciferase activity according to the

manufacturer's instructions (Luciferase Assay System kit, Promega, Madison, WI) with Microplate luminometer LB96V (Perkin-Elmer, Foster City, CA).

Estimation of Log P. The relative hydrophobicity was assessed for each of the rosmarinic acid and its derivatives using a typical method involving measuring the partitioning of the compound between 1-octanol and distilled water. HPLC-grade 1-octanol was pre-saturated with distilled water, and aqueous phase distilled water was saturated with 1-octanol before use. The derivatives were each dissolved in 1-octanol/water phase at final concentration of 1 mM, and an equal volume of 1-octanol/water was added. The tubes were then vortexed continuously for 30 min. The final concentration of compound in both 1-octanol and aqueous phases was measured by HPLC with C₁₈ column. The partition coefficient, P, was determined by dividing the concentration of the derivative in 1-octanol by the concentration in the aqueous phase.

Serum stability. 1 mL of 25% mouse serum/RPMI media (v/v) in 1.5 mL Eppendorf tube was temperature-equilibrated at 37 °C for 15 mins before adding 10 mg/mL of peptide stock solution in RPMI1640 to make the final peptide concentration 100 $\mu\text{g}/\text{mL}$. The initial time was recorded and 100 μL of reaction solution was removed at known time intervals and added to 100 μL of 10% aqueous TCA solution. The reaction sample was cooled 4 °C for 15 mins and spun at 13,000 g for 15 mins to precipitate serum protein. The analysis for the remaining peptide was carried out by reverse phase HPLC using analytical C₁₈ column. Kinetic analysis for the half-life was carried out by a linear least square analysis of the logarithm of the peak area versus time.

Acknowledgements. This work was supported by grant from Inha university.

References

- (a) Cantley, L. C.; Auger, K. R.; Carpenter, C.; Duckworth, B.; Graziani, A.; Kapeller, R.; Soltoff, S. *Cell* **1991**, *64*, 241. (b) Botfield, M. C.; Green, J. *Ann. Rep. Med. Chem.* **1995**, *30*, 227. (c) Pawson, T.; Gish, G. D.; Nash, P. *Trends Cell Biol.* **2001**, *11*, 504.
- (a) Broadbridge, R. J.; Sharma, R. P. *Current Drug Targets* **2000**, *1*, 365. (b) Beaulieu, P. L.; Cameron, D. R.; Ferland, J. M.; Gauthier, J.; Ghio, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Llinas-Brunet, M.; Betageri, R.; Cardozo, M.; Hickey, E. R.; Ingraham, R.; Jakes, S.; Kabcenell, A.; Kirrane, T.; Lukas, S.; Patel, U.; Proudfoot, J.; Sharma, R.; Tong, L.; Moss, N. *J. Med. Chem.* **1999**, *42*, 1757. (c) Rye, C. S.; Baell, J. B. *Curr. Med. Chem.* **2005**, *26*, 3127. (d) Costantio, L.; Barlocco, D. *Curr. Med. Chem.* **2004**, *20*, 2725.
- (a) Vu C. B. *Curr. Med. Chem.* **2000**, *10*, 1081. (b) Garcia-Echeverria, C. *Curr. Med. Chem.* **2001**, *13*, 1589. (c) Sawyer, T. K. *Biopolymers* **1998**, *47*, 243. (d) Sony, Y. L.; Peach, M. L.; Roller, P. P.; Qiu, S.; Wang, S.; Long, Y. Q. *J. Med. Chem.* **2006**, *49*, 1585.
- (a) Shakespeare, W.; Yang, M.; Bohacek, R.; Cerasoli, F.; Stebbins, K.; Sundaramoorthi, R.; Azimioara, M.; Vu, C.; Pradeepan, S.; Metcalf III, C.; Haraldson, C.; Merry, T.; Dalgarno, D.; Narula, S.; Hatada, M.; Lu, X.; Schravendijk, M.; Adams, S.;

- Violette, S.; Smith, J.; Guan, W.; Bartlett, C.; Herson, L.; Iuliucci, J.; Weigele, M.; Sawyer, T. K. *Proc. Natl. Acad. Sci. USA* **2000**, *17*, 9373. (b) Lee, T. R.; Lawrence, D. S. *J. Med. Chem.* **2000**, *43*, 1173. (c) Park, S.; Won, J.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2711. (d) Kang, S.; Park, S.; Shim, H.; Lee, K. H. *Bull. Korean Chem. Soc.* **2003**, *24*, 664.
5. Won, J.; Hur, E. M.; Hur, Y.; Park, S.; Kang, M.; Choi, Y.; Park, C.; Lee, K. H.; Yun, Y. *Eur. J. Immunology* **2003**, *33*, 870.
6. (a) Hur, Y.; Yun, Y.; Won, J. *J. Immunol.* **2004**, *172*, 79. (b) Kang, M. A.; Yun, S. Y.; Won, J. *Blood* **2003**, *101*, 3534.
7. Petersen, M.; Simmonds, M. S. *Phytochemistry* **2003**, *62*, 121.
8. (a) Bogucki, D. E.; Charlton, J. L. *Can. J. Chem.* **1997**, *75*, 1783. (b) Eicher, T.; Ott, M.; Speicher, A. *Synthesis* **1996**, 755.
9. Nakazawa, T.; Ohsawa, K. *J. Nat. Prod.* **1998**, *61*, 993.
10. Park, S. H.; Kang, S. H.; Lim, S. H.; Oh, H. S.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3455.
11. Lee, T. R.; Lawrence, D. S. *J. Med. Chem.* **1999**, *42*, 784.
12. Choi, Y. B.; Kim, C. K.; Yun, Y. *J. Immunol.* **1999**, *163*, 5242.
13. (a) Nasal, A.; Siluk, D.; Kaliszan, R. *Curr. Med. Chem.* **2003**, *10*, 381. (b) Valko, K.; My Du, D.; Bevan, D.; Reynolds, D. P.; Abraham, M. H. *Curr. Med. Chem.* **2001**, *8*, 1137.
14. Powell, M. F.; Stewart, T., Jr.; Otvos, L.; Urge, L.; Gaeta, F. C. A.; Sette, A.; Arrhenius T. *Pharm. Res.* **1993**, *10*, 1268.
15. Hong, S. Y.; Oh, J. E.; Lee, K. H. *Biochem. Pharmacol.* **1999**, *58*, 1775.
16. Zacharie, B.; Lagraoui, M.; Dimarco, M.; Penney, C. L.; Gagnon, L. *J. Med. Chem.* **1999**, *42*, 2046.
-