

# Synthesis of Biologically Active Chalcones and their *Anti-inflammatory* Effects<sup>†</sup>

Jae-Ho Jeon,<sup>‡</sup> Si-Jun Kim,<sup>§</sup> Cheol Gi Kim,<sup>§</sup> Jin-Kyung Kim,<sup>#</sup> and Jong-Gab Jun<sup>‡,\*</sup>

<sup>‡</sup>Institute of Natural Medicine, <sup>§</sup>Department of Chemistry and Institute of Applied Chemistry, Hallym University, Chuncheon 200-702, Korea. \*E-mail: jgjun@hallym.ac.kr

<sup>#</sup>Department of Biomedical Science, College of Natural Science, Catholic University of Daegu, Gyeongsan-Si 700-702, Korea  
Received December 6, 2011, Accepted January 7, 2012

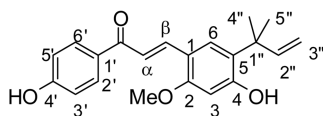
Chalcones have been reported to have various biological activities including antitumor, antiparasitic, anti-leishmanial, antioxidative, superoxide scavenging, antibacterial, and PTP1B activity. Due to the limited natural resources, we had to prepare sizable quantities of biologically active chalcones for bio-tests. Therefore, Claisen-Schmidt condensation between substituted acetophenones and corresponding aldehydes enabled us to prepare chalcones for inflammatory studies. Chalcones thus prepared showed significant suppression of nitric oxide (NO) production at 10  $\mu$ M.

**Key Words :** Chalcones, Claisen-Schmidt condensation, *Anti-inflammatory* studies, EDDA, NO production

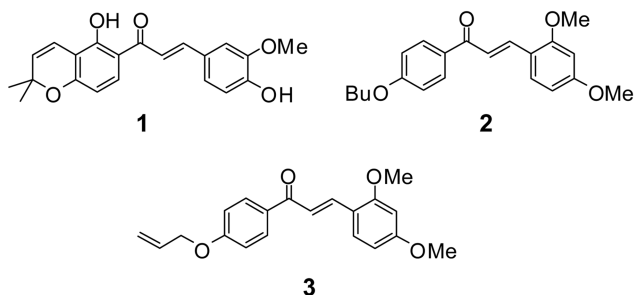
## Introduction

It has been known that chalcones are important precursors for flavonoid biosynthesis.<sup>1</sup> Chalcones, active ingredient of the extracts from the roots of the *Glycyrrhiza inflata* (licorice), draw much attention due to their remarkable biological properties including chemo preventive,<sup>2</sup> antibacterial,<sup>3</sup> antimalarial<sup>4</sup> and antispasmodic activities.<sup>5</sup> Licochalcone A was reported to have significant antitumor activity in various malignant human cell lines (Fig. 1).<sup>2</sup> As we had needed sizable amounts of licochalcone A for *in vitro* tests, we developed a method for efficient preparation of this compound through water-accelerated [3,3]-sigmatropic rearrangement reaction of corresponding aryl prenyl ether system.<sup>6</sup>

One of natural chalcones named 3-methoxy-4-hydroxyonchocarpin (**1**), isolated from the roots of *Lonchocarpus*



**Figure 1.** Structure of licochalcone A.



**Figure 2.** Natural and unnatural chalcones having important biological activities.

*utilis*, was known to be as inhibitors of electron transport acting at NADH (ubiquinone oxidoreductase activity).<sup>7</sup> Despite years passed, malaria is still considered as one of the most deadly diseases affecting eastern Asia and Africa.<sup>4</sup> It is known that 2,4-dimethoxy-4'-butoxychalcone (**2**) has outstanding antimalarial activity.<sup>8</sup> Also, it is known that diseases ranging from the cutaneous healing skin lesions can be cured by licochalcones. 2,4-Dimethoxy-4'-(2-propenyl)oxychalcone (**3**) is known to have antileishmanial activity.<sup>7</sup> Even though the synthesis of chalcone **1** was already reported as shown in Scheme 1,<sup>9</sup> the inflammatory studies are not extensively studied, yet. Chalcone **1** was prepared by using selenium-based solid-phase synthesis with condensation of substituted aldehydes in basic condition (NaOMe/MeOH/THF) followed by deprotection of THP group and oxidation of selenide to selenoxide (Scheme 1).<sup>9</sup>

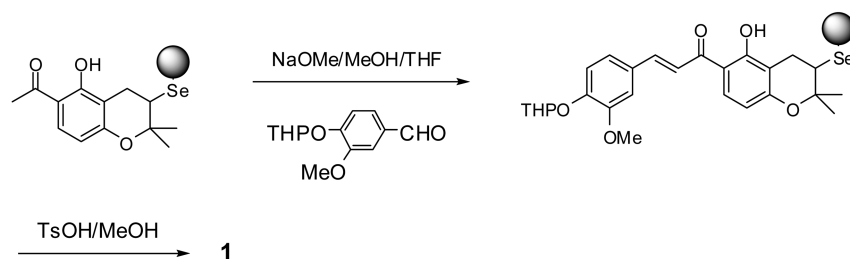
## Results and Discussion

3-Methoxy-4-hydroxyonchocarpin (**1**) was prepared as shown in Scheme 2. The benzopyran **6** was prepared from the ring formation reaction of phenol **4** with 3-methyl-2-butenal (**5**) in the presence of catalytic amount of ethylenediamine diacetate (EDDA) as reported previously.<sup>10</sup> Claisen-Schmidt condensation using KOH/EtOH<sup>11</sup> between **6** and **7** produced **8** in 47% yield. Deprotection of the ethoxymethoxy group with conventional aqueous HCl yielded the product **1** with a low yield (30%) due to workup process. Deblocking using Dowex 50X2 resin yielded the chalcone **1** with an enhanced yield (68%).

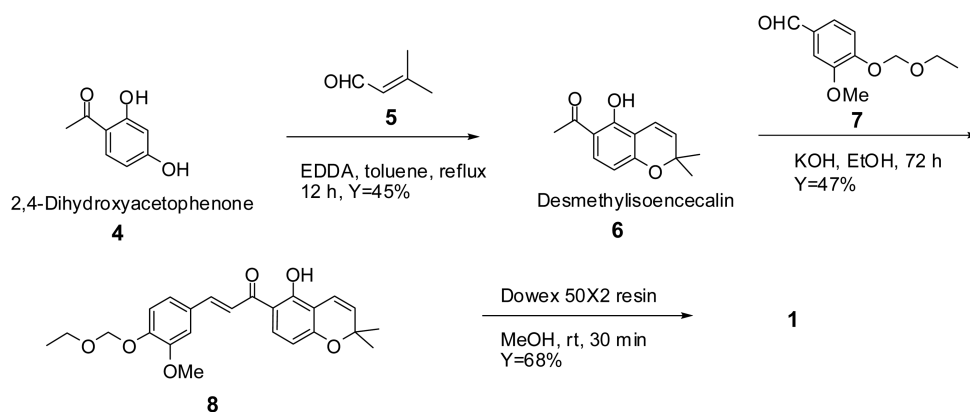
Chalcones **2-3** were prepared by conventional Claisen-Schmidt condensation of substituted acetophenones (**9-10**) with 2,4-dimethoxybenzaldehyde (**11**) using basic condition (NaOH in EtOH) in moderate yields (Scheme 3).

Inflammation is a process of host response to a foreign challenge or tissue injury that leads to the restoration of normal tissue structure and function.<sup>12</sup> In order to study the

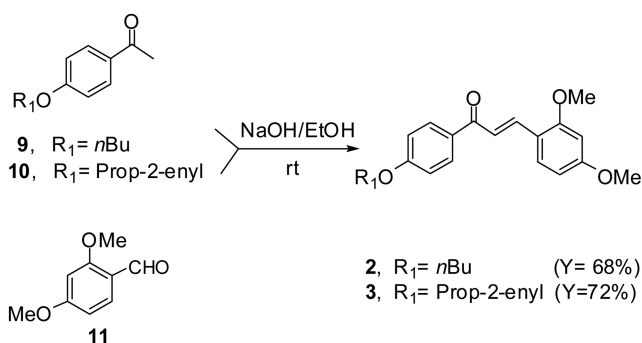
<sup>†</sup>This paper is to commemorate Professor Kook Joe Shin's honourable retirement.



**Scheme 1.** Reported synthesis of 3-methoxy-4-hydroxylonchocarpin (**1**).



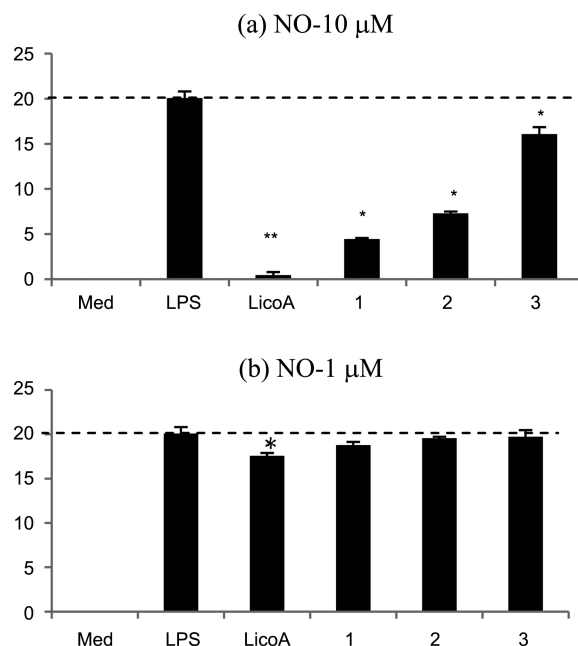
**Scheme 2.** Synthesis of 3-methoxy-4-hydroxylonchocarpin (**1**).



**Scheme 3.** Syntheses of chalcones **2-3**.

*anti*-inflammatory properties of the synthesized chalcones **1-3**, we measured the amount of NO (nitric oxide) in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages (Licochalcone A, abbreviated LicoA was selected as a control group). All of the chalcones tested (licoA, chalcones **1-3**) were proved to have significant suppression of NO production at 10  $\mu\text{M}$  level (Figure 3 and Table 1). The cell viability assay at 10  $\mu\text{M}$  concentration was not affected by the synthetic chalcones **1-3** indicating no cytotoxicity as shown in Figure 4.  $\text{IC}_{50}$  values of synthetic chalcones **1-2** were evaluated by using GraphPad Prism 4.0 software and showed 3.35 and 8.84  $\mu\text{M}$ , respectively (Figure 5).

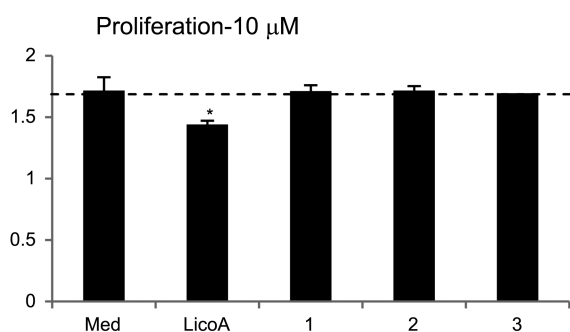
In summary, we prepared chalcones **1-3** by conventional Claisen-Schmidt condensation in basic condition. In *anti*-inflammatory studies, all the compounds tested (**1-3**) showed 77.9%, 63.7% and 19.9% suppression of NO production, respectively, at 10  $\mu\text{M}$  and they proved to have *anti*-inflammatory effects. The precise mechanism of inhibitory action is now under investigation.



**Figure 3.** Effects of chalcones **1-3** on LPS-induced NO production. RAW 264.7 cells were treated with (a) 10  $\mu\text{M}$  and (b) 1  $\mu\text{M}$  of chalcones in the presence of 1  $\mu\text{g mL}^{-1}$  of LPS. Statistical significance is based on the difference when compared with LPS-stimulated cells (\* $P < 0.05$ , \*\* $P < 0.01$ ).

## Experimental

All chemicals were purchased from Sigma-Aldrich Chemicals and were used without further purification unless noted otherwise. NMR spectra were recorded at Varian Mercury-300 MHz FT-NMR and 75 MHz for  $^{13}\text{C}$ , with the chemical

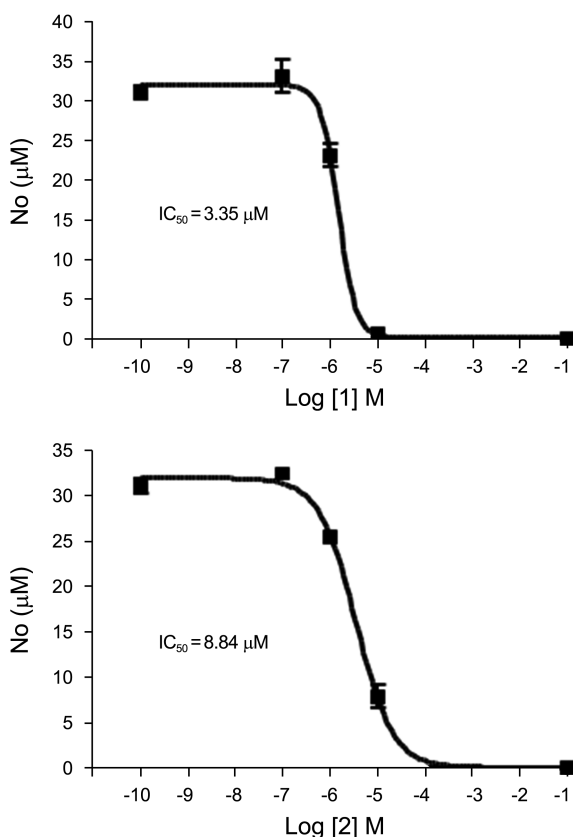


**Figure 4.** Cell viability assay of synthetic chalcones **1-3** at 10  $\mu\text{M}$  concentration. Statistical significance is based on the difference when compared with LPS-stimulated cells (\* $P < 0.05$ ).

**Table 1.** Anti-inflammatory activities of synthetic chalcones **1-3**

Compound	NO Production	(% Inhibition)
	10 $\mu\text{M}$	1 $\mu\text{M}$
LicoA	2.2 $\pm$ 0.3 (97.8)	87.5 $\pm$ 0.3 (12.5)
<b>1</b>	22.1 $\pm$ 0.1 (77.9)	93.5 $\pm$ 0.4 (6.5)
<b>2</b>	36.3 $\pm$ 0.2 (63.7)	97.5 $\pm$ 0.1 (2.5)
<b>3</b>	80.1 $\pm$ 0.8 (19.9)	98.2 $\pm$ 0.8 (1.8)
LPS	100.0 $\pm$ 0.7 (0.0)	100.0 $\pm$ 0.7 (0.0)

The results are reported as mean value  $\pm$  SEM for  $n = 3$ . % Inhibition is based on LPS as shown in parenthesis.



**Figure 5.**  $\text{IC}_{50}$  values of synthetic chalcones **1-2**.

shift ( $\delta$ ) reported in parts per million (ppm) relative to TMS and the coupling constants ( $J$ ) quoted in Hz.  $\text{CDCl}_3$  was used as a solvent and an internal standard. Thin-layer

chromatography (TLC) was performed on DC-Plastikfolien 60, F<sub>254</sub> (Merck, layer thickness 0.2 mm) plastic-backed silica gel plates and visualized by UV light (254 nm) or staining with *p*-anisaldehyde. 2,4-Dihydroxyacetophenone (**4**), 3-methyl-2-butenal (**5**) and 2,4-dimethoxybenzaldehyde (**11**) were commercially available from Sigma-Aldrich Chemicals. LPS derived from *Escherichia coli* and dimethylsulfoxide (DMSO) was obtained from Sigma (St Louis, Mo, USA). The Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin used in this study were obtained from Hyclone (Logan, Utah, USA). The final concentrations of DMSO never exceeded 0.1%, which did not affect the assay systems.

**Cell Culture and Cell Viability Assay.** RAW264.7 murine macrophages were obtained from the Korean Cell Bank (Seoul, Korea) and cultured in DMEM containing 10% FBS, 100 U/mL penicillin, and 100  $\mu\text{g}/\text{mL}$  streptomycin at 37  $^\circ\text{C}$  in 5%  $\text{CO}_2$ . The effects of chalcones prepared (**1-3**) on cell viability were tested using the CellTiter 96<sup>®</sup> Aqueous One Solution Assay of cell proliferation (Promega, Madison, WI, USA), which uses colorimetry to count the number of viable cells. This assay was used to determine the number of viable cells remaining after the culturing process was complete. RAW264.7 cells were plated at a density of  $2 \times 10^4$  cells in a 96-well flat-bottom plate, and the chalcones prepared were added to each plate at indicated concentrations 0, 1 and 10  $\mu\text{M}$ . After a 24 h incubation period, the number of viable cells was counted according to the manufacturer's instructions. This assay is based on the reduction of a tetrazolium compound, MTS, to formazan, which has an optimum absorption at 490 nm. Thus, the quantity of the product in the cell culture is indicated by the optical density of formazan at 490 nm, which is directly proportional to the number of living cells.

**Measurement of NO.** The amount of NO produced by the mouse macrophage was indicated by the amount that was measured in the RAW264.7 cell culture supernatant. RAW264.7 cells were plated at a density of  $5 \times 10^5$  cells in a 24-well cell culture plate with 500  $\mu\text{L}$  of culture medium and incubated for 12 h. They were then treated with 1 or 10  $\mu\text{M}$  of each compound in 1  $\mu\text{g}/\text{mL}$  of LPS and incubated for another 18 h. The amount of NO produced was measured using the Griess reagent system (Promega). The values were evaluated by one-way analysis of variance (ANOVA) with Bonferroni multiple comparison post tests using the GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA). The  $\text{IC}_{50}$  value was estimated by using GraphPad Prism 4.0 software.

**Desmethyloencecalin (6).** This compound was prepared according to the reported procedure.<sup>10</sup> In a 2-neck round bottom flask, 2,4-dihydroxyacetophenone (**4**) (456 mg, 3.0 mmol) and 3-methyl-2-butenal (**5**) (433  $\mu\text{L}$ , 4.5 mmol), ethylenediamine diacetate (54 mg, 0.3 mmol) was placed in toluene (50 mL) and refluxed for 12 h. The reaction flask was cooled down and toluene was evaporated *in vacuo*. To the reaction mixture, ethyl acetate (50 mL) was added and the organic phase was washed with deionized distilled water

(30 mL × 2). The organic phase was separated and dried over anhydrous MgSO<sub>4</sub> and filtered. Concentration *in vacuo* and the crude reaction mixture was purified by *silica* gel column chromatography (EtOAc/hexane=1/10) to give a yellowish solid (294 mg, 45%). *R<sub>f</sub>* 0.50 (EtOAc/hexane=1/6); mp 98-100 °C (lit. mp 101-103 °C); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.49 (d, *J* = 8.7 Hz, 1H), 6.69 (d, *J* = 10.2 Hz, 1H), 6.31 (d, *J* = 8.7 Hz, 1H), 5.56 (d, *J* = 10.2 Hz, 1H), 2.50 (s, 3H), 1.44 (s, 6H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 202.4, 159.5, 159.4, 131.5, 128.0, 115.6, 113.7, 109.1, 108.2, 77.6, 28.3, 28.1.

**3-(4-Ethoxymethoxy-3-methoxyphenyl)-1-(5-hydroxy-2,2-dimethyl-2*H*-chromen-6-yl)propenone (8).** In one neck round bottom flask (20 mL), dissolved mixture of **6** (106 mg, 0.49 mmol) with **7** (153 mg, 0.73 mmol) <sup>13</sup> in ethanol (5 mL) was added KOH (136 mg, 2.43 mmol) at rt. The reaction mixture was stirred for 72 h at rt. After concentration of solvent under reduced pressure, ether (30 mL) and 3 N HCl (20 mL) were added to turn acidic. Phase separation, addition of saturated sodium bicarbonate solution (20 mL), washing with brine (20 mL), separation of the ether layer, drying over anhydrous sodium sulfate, filtering, concentration *in vacuo* and purification by *silica* gel column chromatography (EtOAc/hexane=1/5) gave a yellow oil (94 mg, 47%). *R<sub>f</sub>* 0.20 (EtOAc/hexane=1/6); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.82 (d, *J* = 15.9 Hz, 1H), 7.71 (d, *J* = 9.0 Hz, 1H), 7.42 (d, *J* = 15.9 Hz, 1H), 7.21 (d, *J* = 9.5 Hz, 2H), 7.14 (s, 1H), 6.74 (d, *J* = 10.5 Hz, 1H), 6.37 (d, *J* = 9.0 Hz, 1H), 5.58 (d, *J* = 9.6 Hz, 1H), 5.33 (s, 2H), 3.95 (s, 3H), 3.77 (q, *J* = 6.9 Hz, 2H), 1.42 (s, 6H), 1.23 (t, *J* = 6.9 Hz, 3H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 191.6, 160.8, 159.6, 149.7, 149.0, 144.2, 130.5, 128.9, 128.0, 122.7, 118.4, 115.8, 115.7, 114.0, 110.9, 109.4, 108.1, 93.8, 77.8, 64.6, 56.1, 28.4, 15.2.

**3-Methoxy-4-hydroxyonchocarpin (1).** Dowex 50X2 cation exchange resin (70 mg) was added to the compound **8** (50 mg, 0.12 mmol) in MeOH (2 mL) and stirred at rt for 30 min. The Dowex resin was filtered and MeOH was concentrated *in vacuo*, and the crude reaction mixture was purified by *silica* gel column chromatography (EtOAc/hexane=1/3) to give a yellow oil (29 mg, 68%). The spectral data for this compound agreed well with the literature values.<sup>9</sup> *R<sub>f</sub>* 0.10 (EtOAc/hexane=1/6); <sup>1</sup>H NMR (300 MHz, acetone-*d*<sub>6</sub>) δ 8.00 (d, *J* = 8.7 Hz, 1H), 7.85 (d, *J* = 15.3 Hz, 1H), 7.74 (d, *J* = 15.3 Hz, 1H), 7.50 (s, 1H), 7.30 (d, *J* = 6.9 Hz, 1H), 6.87 (d, *J* = 6.9 Hz, 1H), 6.68 (d, *J* = 10.2 Hz, 1H), 6.34 (d, *J* = 8.7 Hz, 1H), 5.71 (d, *J* = 10.2 Hz, 1H), 3.95 (s, 3H), 1.42 (s, 6H). <sup>13</sup>C NMR (75 MHz, acetone-*d*<sub>6</sub>) δ 192.5, 161.0, 160.8, 151.8, 148.7, 145.8, 131.6, 128.7, 126.5, 125.1, 116.9, 116.0, 115.8, 114.5, 111.5, 109.4, 108.3, 78.0, 55.9, 28.5.

**2,4-Dimethoxy-4'-butoxychalcone (2).** The acetophenone **9** (41 mg, 0.213 mmol)<sup>7,8</sup> and the aldehyde **11** (35 mg, 0.213 mmol) were reacted in EtOH (5 mL) with NaOH (26 mg, 0.426 mmol) at rt. After 48 h, EtOH was concentrated *in vacuo* and the crude reaction mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The organic phase was washed by deionized distilled water (10 mL × 2), dried over anhydrous MgSO<sub>4</sub>,

concentrated *in vacuo*, and purified by flash *silica* gel column chromatography (acetone/hexane=1/9) to give a yellow liquid (49 mg, 68%). The spectral data for this compound agreed well with the literature values.<sup>7</sup> *R<sub>f</sub>* 0.27 (acetone/hexane=1/5); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.02 (d, *J* = 15.6 Hz, 1H), 8.10-7.97 (m, 2H), 7.56 (d, *J* = 15.6 Hz, 1H), 7.51 (d, *J* = 7.5 Hz, 1H), 6.92 (dd, *J* = 8.7, 1.8 Hz, 2H), 6.50 (dd, *J* = 9.3, 2.4 Hz, 1H), 6.45 (d, *J* = 2.4 Hz, 1H), 4.02 (t, *J* = 6.3 Hz, 2H), 3.88 (s, 3H), 3.85 (s, 3H), 1.83-1.73 (m, 2H), 1.56-1.44 (m, 2H), 0.98 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 189.1, 162.6, 162.5, 160.1, 139.3, 131.3, 130.6, 130.5, 120.2, 117.3, 114.0, 105.3, 98.4, 67.9, 55.5, 55.5, 31.2, 19.3, 13.9.

**2,4-Dimethoxy-4'-(2-propenyloxy)chalcone (3).** The acetophenone **10** (66 mg, 0.375 mmol)<sup>14,15</sup> and the aldehyde **11** (62 mg, 0.375 mmol) were reacted in EtOH (3 mL) with NaOH (30 mg, 0.750 mmol) at rt. After 48 h, EtOH was concentrated *in vacuo* and the crude reaction mixture was extracted by CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The organic phase was washed by deionized distilled water (10 mL × 2), dried over anhydrous MgSO<sub>4</sub>, concentrated *in vacuo*, and purified by flash *silica* gel column chromatography (acetone/hexane=1/9) to give a yellow liquid (83 mg, 72%). The spectral data for this compound agreed well with the literature values.<sup>9,14</sup> *R<sub>f</sub>* 0.21 (acetone/hexane=1/5); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.02 (d, *J* = 15.6 Hz, 1H), 7.80 (dd, *J* = 6.0, 2.1 Hz, 2H), 7.54 (d, *J* = 15.6 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 1H), 6.83 (dd, *J* = 6.0, 2.1 Hz, 2H), 6.52 (dd, *J* = 9.0, 2.1 Hz, 1H), 6.47 (d, *J* = 5.1 Hz, 1H), 6.11-5.99 (m, 1H), 5.42 (dd, *J* = 15.9, 1.0 Hz, 1H), 5.31 (dd, *J* = 10.2, 1.5 Hz, 1H), 4.60 (dd, *J* = 3.0, 1.5 Hz, 2H), 3.89 (s, 3H), 3.84 (s, 3H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 189.1, 162.6, 161.9, 160.1, 139.5, 132.5, 131.7, 130.7, 130.5, 120.1, 118.0, 117.2, 114.3, 105.3, 98.4, 68.8, 55.5, 55.5.

**Acknowledgments.** This research was financially supported by the Ministry of Education, Science Technology (MEST) and Korea Institute for Advancement of Technology (KIAT) through the Human Resource Training Project for Regional Innovation (4R09-0301-003-S000100), and by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0030750).

## References

- Cushine, T. P. T.; Lamb, A. J. *Int. J. Antimicrob. Ag.* **2005**, *26*, 343.
- Shibata, S. *Stem Cells* **1994**, *12*, 44.
- (a) Liu, X. L.; Xu, Y. J.; Go, M. L. *Eur. J. Med. Chem.* **2008**, *43*, 1681. (b) Kwon, H. S.; Park, J. H.; Kim, D. H.; Kim, Y. H.; Park, J. H.; Shin, H. K.; Kim, J. K. *J. Mol. Med. (Berl)* **2008**, *86*, 1287.
- Liu, M.; Wilarirar, P.; Go, M.-L. *J. Med. Chem.* **2001**, *44*, 4443.
- Nagai, H.; He, J.-X.; Tani, T.; Akao, T. *J. Pharm. Pharmacol.* **2007**, *59*, 1421.
- Jeon, J.-H.; Kim, M. R.; Jun, J.-G. *Synthesis* **2011**, 370.
- Nielsen, S. F.; Christensen, S. B.; Cruciani, G.; Kharazmi, A.; Liljefors, T. *J. Med. Chem.* **1998**, *41*, 4819.
- Chen, M.; Christensen, S. B.; Zhai, L.; Rasmussen, M. H.; Theander,

- T. G.; Frokjaer, S.; Steffansen, S.; Davidsen, J.; Kharazmi, A. *J. Infect. Dis.* **1997**, *176*, 1327.
9. (a) Nicolaou, K. C.; Pfefferkorn, J. A.; Cao, G-Q. *Angew. Chem. Int. Ed.* **2000**, *39*, 734. (b) Nicolaou, K. C.; Pfefferkorn, J. A.; Roecker, A. J.; Cao, G-Q.; Barluenga, S.; Mitchell, H. J. *J. Am. Chem. Soc.* **2000**, *122*, 9939.
10. Lee, Y. R.; Wang, X.; Xia, L. *Molecules* **2007**, *12*, 1420.
11. (a) Claisen, L.; Claparede, A. *Ber.* **1881**, *14*, 2460. (b) Schmidt, J. *G. Ber.* **1881**, *14*, 1459.
12. Vermooy, J. H.; Dentener, M. A.; Suylen, R. J. van.; Buurman, W. A.; Wouters, E. F. *Am. J. Respir Cell Mol. Biol.* **2002**, *26*, 152.
13. (a) Nabaei-Bidhendi, G.; Bannerjee, N. R. *J. Ind. Chem. Soc.* **1990**, *67*, 43. (b) Ruffin, B.; Castellan, A.; Grelier, S.; Nourmamode, A.; Riela, S.; Trichet, V. *J. Appl. Poly. Sci.* **1998**, *69*, 2517.
14. Kharazmi, A.; Christensen, S. B.; Ming, C.; Theander, T. G. US 5,985,935, 1999.
15. Claisen, L.; Eisleb, O. *Justus Liebigs Ann. Chem.* **1913**, *401*, 21.
-