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Preparation of Benzoyloxy Benzophenone Derivatives and Their Inhibitory Effects of ICAM-1 Expression

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Benzoyloxy benzophenone derivatives were prepared in 3 steps including DCC coupling, Fries rearrangement and esterification from benzoic acids in 24-89% total yields. Among the prepared 12 benzophenone analogues **1a-1l**, the compound **1b** having three chloro groups at the para position showed maximum inhibitory effects of ICAM-1 expression but, **1a** which have no substituents at all showed no inhibitory activity. This study provides the evidences that benzoyloxy benzophenone derivative, **1b** may exert its anti-inflammatory activity by suppressing IFN-γ-induced ICAM-1 expression.

Key Words : Benzoyloxy benzophenone, ICAM-1, Lewis acid, Fries rearrangement, Regioselectivity

Introduction

Inflammation is a complex biological process involved in the development of various skin diseases such as atopic dermatitis.[1] Up-regulation of adhesion molecules and cytokines/chemokines may induce a series of interactions between monocytes, endothelial cells, and keratinocytes, resulting in infiltration of leukocytes into the area of inflamed skin.[2] Increased expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) on the surface of epidermal keratinocytes as well as dermal microvascular endothelial cells has been proposed as an important regulator in the inflammatory immune responses. The epidermal keratinocyte, one of major cell type in the skin, can express ICAM-1 in the response to cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α).[3] Modulation of ICAM-1 expression in the epidermal keratinocyte provides a rationale for development of anti-inflammatory compounds that may be useful for various inflammatory skin diseases. In continuation of our drug discovery program on nonsteroidal anti-inflammatory drugs (NSAIDs) we wanted to find compounds which suppress IFN-γ-induced expression of ICAM-1.

Benzophenone analogues were known to have anti-inflammatory activity[4] and since then many derivatives have been synthesized as effective anti-inflammatory agents by several scientists.[5] Recently, benzoyloxy benzophenones were reported as potent anti-inflammatory agent by modifying the hydroxyl group of hydroxybenzophenones with incorporation of benzoyloxy group.[6] In this report, we describe the syntheses of 12 benzoyloxy benzophenone derivatives **1** using practical methods and their anti-inflammatory activities *via* inhibitory effects on IFN-γ-induced expression of ICAM-1 (Figure 1).

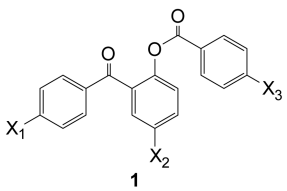
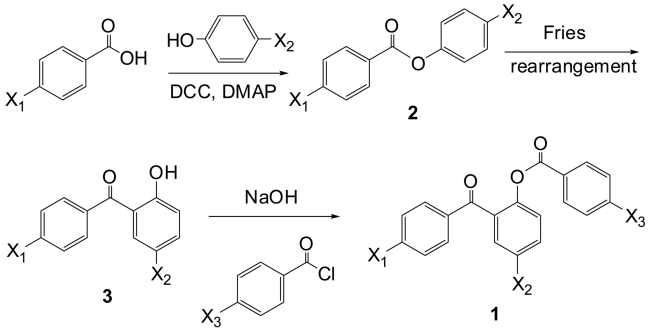


Figure 1. Structure of benzoyloxy benzophenone.

Results and Discussion

Among benzoyloxy benzophenone derivatives reported, the compound **1** having three chloro groups at the para position (X_1=X_2=X_3=Cl; **1b**) showed maximum inhibition in anti-inflammatory activity by suppressing cyclooxygenase (COX) activity.[6] These results prompted us to synthesize benzoyloxy benzophenone derivatives having other halogens or alkoxy substituents at the para position in the structure for the comparison of their anti-inflammatory activities on the other inflammatory responses than COX 2 inhibition. The synthesis of benzoyloxy benzophenone derivatives is straightforward as shown in Scheme 1, which includes DCC coupling of benzoic acid with phenol, Fries rearrangement of ester **2** to aryl ketone **3**, and esterification of phenol **3** to



Scheme 1. Synthesis of benzoyloxy benzophenone.

the product **1**.

Formation of aryl ester **2** is obtained with almost quantitative yields using DCC coupling of benzoic acid with phenol derivatives. Fries rearrangement is well documented, however, the regioselectivity between the formation of 2- or 4-hydroxybenzophenones is still on question because of the versatility on substituents in the structure, which results a low yield, too.[7] At temperature below 100 °C the para isomer is normally formed preferentially, but above this temperature the *ortho* isomer predominates. We used different Lewis acids with high boiling solvents such as xylene, nitrobenzene and DMF for the Fries rearrangement reaction to find the best condition producing only *ortho*-hydroxybenzophenone with high yield (Table 1). The use of $FeCl_3$ (entry 4) or MsOH (entry 5) as a Lewis acid with xylene showed a low yield of *ortho*-product **3a** and other Lewis acids including $ZnCl_2$, $InCl_3$, $BF_3{\cdot}OEt_2$ and *p*-TsOH (entries 6-9) yielded no product. Of the many methods available for the Fries rearrangement reaction, the most widely used Lewis acid is $AlCl_3$ and the solvent is nitrobenzene, however, the high boiling nitrobenzene as a solvent is the most difficult to remove using usual methods in our hand (entry 3). Changing the solvent to xylene (entry 2) in this reaction allowed only the *ortho*-product **3a** in 64% yield, and finally to DMF (entry 1) in 99% yield. So, we set the Fries rearrangement condition up to use $AlCl_3$ as a Lewis acid and DMF as a solvent at 210 °C in a sealed tube within 3 h to give the *ortho*-products **3a-3l** with 44-99% yield (Table 2).

Table 1. Fries rearrangement of ester **2a** to the *ortho*-benzophenone **3a**

Entry	Lewis acid	Solvent	Temperature	Yield (%)
1	$AlCl_3$	DMF	210 °C	99
2	$AlCl_3$	xylene	160 °C	64
3	$AlCl_3$	nitrobenzene	210 °C	#
4	$FeCl_3$	xylene	160 °C	50
5	MsOH	xylene	160 °C	20
6	$ZnCl_2$	xylene	160 °C	No Rxn
7	$InCl_3$	xylene	160 °C	No Rxn
8	$BF_3{\cdot}OEt$	xylene	160 °C	No Rxn
9	*p*-TsOH	xylene	160 °C	No Rxn

#The solvent nitrobenzene could not be removed in usual methods.

Table 2. Syntheses of benzoyloxy benzophenone derivatives **1**

Entry	Substrate			Yield (%)			
	X_1	X_2	X_3	2	3	1	Total
a	H	H	H	98	99	78	75
b	Cl	Cl	Cl	98	56	86	47
c	Br	Br	Br	98	48	80	37
d	I	I	I	95	62	86	51
e	OMe	OMe	OMe	95	52	83	41
f	OBn	OBn	OBn	96	44	56	24
g	Br	Br	H	98	48	72	34
h	Br	H	Br	96	55	72	38
i	H	Br	H	99	55	82	45
j	H	H	Br	98	99	92	89
k	H	Br	Br	99	55	82	45
l	Br	H	H	96	55	75	40

Following esterification of the *ortho*-benzophenones with 4-substituted acyl chlorides using 10% aqueous NaOH solution in methylene chloride produced the benzoyloxy benzophenones in 56-92% yields. Three steps involving DCC coupling, Fries rearrangement and esterification from benzoic acids gave the benzoyloxy benzophenones **1a-1l** in 24-89% total yields.

The cytotoxicity of selected 4 benzoyloxy benzophenones **1a-1d** was measured by an MTT assay in HaCaT cells. As shown in Figure 2, cell viability was not significantly affected by 4 benzoyloxy benzophenones **1a-1d** up to 20 μM, indicating no significant cytotoxicity at this concentration. The other benzoyloxy benzophenones **1e-1l** showed no significant cytotoxic effects at the concentration of 20 μM (data not shown). In order to investigate the anti-inflammatory properties of the prepared benzoyloxy benzophenones **1a-1l**, we analyzed the effect of these compounds on IFN-γ-induced ICAM-1 expression in HaCaT cells. HaCaT cells were treated with each compound at 20 mM concentration for 1 h, and stimulated with IFN-γ, and then protein expression of ICAM-1 were measured by Western blot analysis. Among benzoyloxy benzophenone derivatives, **1b** significantly inhibited IFN-γ-induced protein expression of ICAM-1, while other compounds had minimal effects (Fig. 3). To investigate whether benzoyloxy benzophenone derivative, **1b** has inhibitory effects on IFN-γ-induced mRNA expression of ICAM-1, we next analyzed the levels of ICAM-1 mRNA by RT-PCR. As shown in Figure 4, **1b** significantly suppressed IFN-γ-induced mRNA expression of ICAM-1. However, the other benzophenones **1a**, **1c** and **1d** had a minimal effect on ICAM-1 mRNA expression.

In conclusion, 12 benzoyloxy benzophenone derivatives were prepared in 3 steps including DCC coupling, Fries rearrangement and esterification from benzoic acids in 24-89% total yields. Among the prepared benzophenone analogues **1a-1l**, the compound **1b** having three chloro groups at the para position showed maximum inhibitory activity (75%) of ICAM-1 expression. The bromo analogues **1c** and

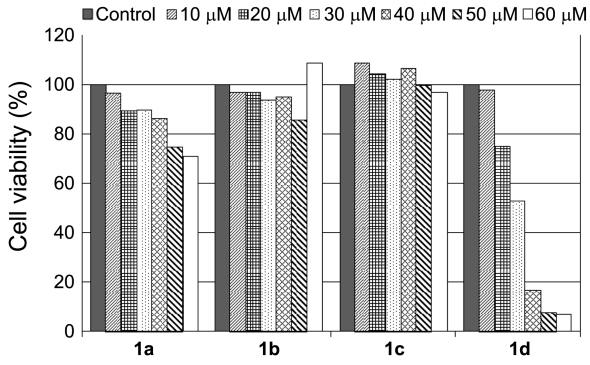


Figure 2. Cytotoxic effects of compounds in HaCaT cells. The cells were treated with each compound at the concentration of 10-60 μM for 24 h and then cell viability was measured by MTT assay.

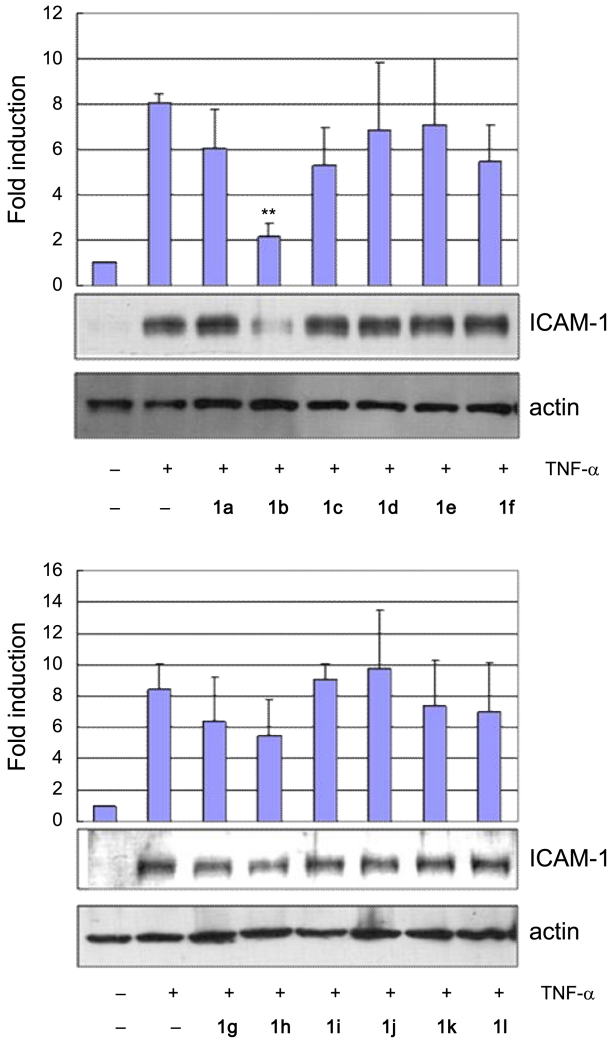


Figure 3. Effects of compounds on IFN-γ-induced ICAM-1 expression. HaCaT cells were pretreated with each compound at 20 μM for 1 h, and then stimulated with IFN-γ (100 U/mL) for 12 h. Total protein was analyzed by Western blot using antibodies against ICAM-1 or β-actin.

1h both showed 35% inhibition each and the benzyloxy substituent **1f** also showed 32% inhibition, but iodo or other substituents at para position of benzophenones showed little or no inhibition effects. This study provides the evidences that benzoyloxy benzophenone derivative, **1b** may exert its anti-inflammatory activity by suppressing IFN-γ-induced ICAM-1 expression.

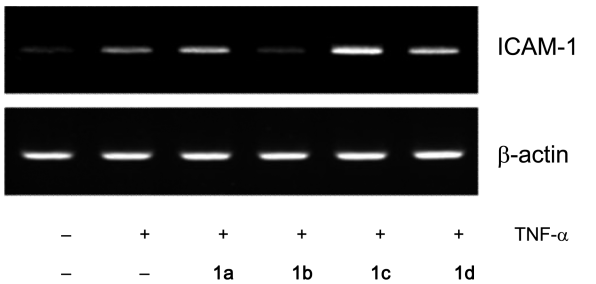


Figure 4. Effects of compounds on IFN-γ-induced mRNA expression of ICAM-1. HaCaT cells were pretreated with each compound at 20 μM for 1 h, and then stimulated with IFN-γ (100 U/mL) for 1 h. The levels of mRNA were analyzed by RT-PCR using the relevant primers.

Experimental

All chemicals used were purchased from commercial sources and used as received unless otherwise stated. NMR spectra were recorded at Varian Mercury-300 MHz FT-NMR for ^{1}H and 75 MHz for ^{13}C, with the chemical shifts (δ) reported in parts per million (ppm) relative to TMS and the coupling constants (*J*) quoted in Hz. $CDCl_3$ was used as a solvent and an internal standard. LC-MS spectra were measured on a Thermo Finnigan LCQ Advantage Max System. Flash chromatography was carried out using silica gel Merck 60 (230-400 mesh). Thin-layer chromatography (TLC) was performed on DC-Plastikfolien 60, F_{254} (Merck, layer thickness 0.2 mm) plastic-backed silica gel plates with visualization by UV light (254 nm) or by treatment with *p*-anisaldehyde. Melting points were measured on a MEL-TEMP II apparatus and were uncorrected.

General Procedure of DCC Coupling Reaction.

Phenyl Benzoate (2a): Benzoic acid (2.00 g, 16.38 mmol) was dissolved in dry CH_2Cl_2 (100 mL) under N_2 atmosphere and phenol (1.54 g, 16.38 mmol), DCC (5.07 g, 24.55mmol), DMAP (0.40 g, 8.19mmol) was slowly added and stirred for 8 hr at rt. The reaction was quenched by adding water and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, removed by filtration. The filtrate was concentrated *in vacuo* to give a yellow solid. The solid was chromatographed on silica gel to give a white solid (3.18 g, 98%). R_f 0.90 (EtOAc:Hexane = 1:3); mp 57-59 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (2H, d, *J* = 6.9 Hz), 7.61 (1H, t, *J* = 6.9 Hz), 7.49 (2H, t, *J* = 7.5 Hz), 7.41 (2H, t, *J* = 7.8 Hz), 7.25 (1H, 7, *J* = 7.5 Hz), 7.20 (2H, d, *J* = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 151.9, 137.8, 132.2, 130.1, 128.5, 128.0, 125.1, 124.9.

4-Chlorophenyl 4-chlorobenzoate (2b): Yield (98%). R_f 0.81 (EtOAc:Hexane = 1:2); mp 85-87 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (2H, d, *J* = 8.1 Hz), 7.47 (2H, d, *J* = 8.4 Hz), 7.38 (2H, d, *J* = 8.4 Hz), 7.14 (2H, d, *J* = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 149.1, 137.5, 135.9, 131.5, 130.6, 128.8, 128.7, 128.6.

4-Bromophenyl 4-bromobenzoate (2c): Yield (98%). R_f 0.65 (EtOAc:Hexane = 1:2); mp 95-98 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (2H, d, *J* = 8.4 Hz), 7.63 (2H, d, *J* = 8.7 Hz), 7.52 (2H, d, *J* = 8.7 Hz), 7.08 (2H, d, *J* = 9.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.1, 148.3, 133.5, 132.9, 131.0, 130.4, 129.9, 125.6, 120.9.

4-Iodophenyl 4-iodobenzoate (2d): Yield (95%). R_f 0.83 (EtOAc:Hexane = 1:2); mp 155-158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (2H, d, *J* = 8.6 Hz), 7.91 (2H, d, *J* = 9.0 Hz), 7.81 (2H, d, *J* = 8.8 Hz), 7.05 (2H, d, *J* = 8.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.0, 150.0, 137.8, 137.3, 131.7, 129.5, 123.0, 102.5, 94.1.

4-Methoxyphenyl 4-methoxybenzoate (2e): Yield (95%).

R_f 0.73 (EtOAc:Hexane = 1:2); mp 95-97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (2H, d, J = 8.7 Hz), 7.36 (2H, d, J = 8.7 Hz), 7.21 (2H, d, J = 8.7 Hz), 7.11 (2H, d, J = 8.6 Hz), 4.03 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 165.3, 156.9, 146.7, 140.6, 132.1, 126.6, 122.5, 117.2, 56.0, 55.7.

4-Benzyloxyphenyl 4-benzyloxybenzoate (2f): Yield (96%). R_f 0.79 (EtOAc:Hexane = 1:2); mp 102-104 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (2H, d, J = 8.2 Hz), 7.56-7.14 (16H, m), 5.20 (2H, s), 5.04 (2H, s).

4-Bromophenyl 4-bromobenzoate (2g): same compound as **2c**.

Phenyl 4-bromobenzoate (2h): Yield (96%). R_f 0.65 (EtOAc:Hexane = 1:2); mp 70-73 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (2H, d, J = 8.9 Hz), 7.55 (2H, d, J = 8.6 Hz), 7.45 (2H, t, J = 8.3 Hz), 7.37 (2H, d, J = 8.7 Hz), 7.29 (1H, t, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 150.9, 132.5, 130.1, 129.3, 129.0, 128.3, 126.6, 120.7.

4-Bromophenyl benzoate (2i): Yield (99%). R_f 0.85 (EtOAc:Hexane = 1:2); mp 79-82 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (2H, d, J = 8.4 Hz), 7.69 (1H, t, J = 8.1 Hz), 7.63 (2H, d, J = 8.5 Hz), 7.52 (2H, t, J = 8.3 Hz), 7.18 (2H, d, J = 8.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 148.3, 137.5, 136.9, 134.0, 132.4, 131.9, 130.6, 123.9.

Phenyl benzoate (2j): same compound as **2a**.

4-Bromophenyl benzoate (2k): same compound as **2i**.

Phenyl 4-bromobenzoate (2l): same compound as **2h**.

General Procedure of Fries Rearrangement Reaction.

2-Hydroxybenzophenone (3a): Phenyl benzoate (0.10 g, 0.50 mmol) in DMF (5 mL) was dissolved with $AlCl_3$ (0.07 g, 0.50 mmol). The solution in sealed tube was heated using furnace for 3 hr at 210 °C. The reaction mixture was cooled, mixed with 1 N HCl, extracted with $CHCl_3$, dried over anhydrous $MgSO_4$ and concentrated. The residue was chromatographed on silica gel to give yellow oil (0.10 g, 99%). R_f 0.71 (EtOAc:Hexane = 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.13 (1H, s), 8.15 (2H, d, J = 8.1 Hz), 7.76-7.40 (5H, m), 7.15 (1H, 7, J = 8.1 Hz), 6.95 (1H, d, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.5, 159.5, 137.8, 135.7, 134.6, 131.5, 130.0, 126.5, 123.8, 122.5, 116.5.

2-Hydroxy-4',5-dichlorobenzophenone (3b): Yield (56%). R_f 0.63 (EtOAc:Hexane = 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (2H, d, J = 8.7 Hz), 7.54 (1H, s) 7.37 (2H, d, J = 8.7 Hz), 7.16 (1H, d, J = 8.7 Hz), 6.75 (1H, d, J = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.6, 160.7, 141.5, 137.9, 135.0, 134.0, 131.5, 129.0, 126.1, 125.9, 115.8.

2-Hydroxy-4',5-dibromobenzophenone (3c): Yield (48%). R_f 0.83 (EtOAc:Hexane = 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (1H, s), 7.60 (1H, d, J = 8.1 Hz), 7.52 (2H, d, J = 8.3 Hz), 7.47 (2H, d, J = 8.3 Hz), 6.72 (1H, d, J = 8.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.9, 161.8, 140.9, 136.8, 135.4, 132.3, 132.0, 131.9, 126.9, 125.9, 116.4.

2-Hydroxy-4',5-diiodobenzophenone (3d): Yield (62%). R_f 0.76 (EtOAc:Hexane = 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (1H, s), 7.81 (1H, d, J = 8.3 Hz), 7.79 (2H, d, J = 8.7 Hz), 7.61 (2H, d, J = 8.5 Hz) 6.95 (1H, d, J = 8.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.8, 160.5, 149.4, 137.1, 136.7, 131.7, 126.6, 122.9, 120.8, 101.0, 92.0.

2-Hydroxy-4',5-dimethoxybenzophenone (3e): Yield (52%). R_f 0.80 (EtOAc:Hexane = 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (2H, d, J = 8.5 Hz), 7.34-6.81 (5H, m), 3.99 (3H, s), 3.98 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.1, 170.6, 155.9, 152.5, 140.7, 130.9, 130.3, 121.6, 119.6, 114.8, 110.3, 55.0, 54.8.

2-Hydroxy-4',5-dibenzyloxybenzophenone (3f): Yield (44%). R_f 0.59 (EtOAc:Hexane = 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.00 (1H, s), 7.90-7.00 (17H, m), 5.12 (3H, s), 5.08 (3H, s).

2-Hydroxy-4',5-dibromobenzophenone (3g): same compound as **3c**.

2-Hydroxy-4'-bromobenzophenone (3h): Yield (55%). R_f 0.47 (EtOAc:Hexane = 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (1H, s), 7.70 (2H, d, J = 8.2 Hz), 7.58 (2H, d, J = 8.7 Hz), 7.55 (2H, d, J = 8.7 Hz), 6.89 (1H, d, J = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.7, 169.2, 138.7, 136.9, 133.5, 132.5, 129.8, 128.3, 125.1, 120.5, 115.1.

2-Hydroxy-5-bromobenzophenone (3i): Yield (55%). R_f 0.48 (EtOAc:Hexane = 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92-7.53 (7H, m), 6.91 (1H, d, J = 8.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.8, 160.2, 141.7, 139.4, 136.7, 135.2, 132.3, 128.3, 120.1, 119.8, 116.4.

2-Hydroxybenzophenone (3j): same compound as **3a**.

2-Hydroxy-5-bromobenzophenone (3k): same compound as **3i**.

2-Hydroxy-4'-bromobenzophenone (3l): same compound as **3h**.

General Procedure of Esterification.

2-Benzoyloxy benzophenone (1a): 2-Hydroxybenzophenone (0.10 g, 0.50 mmol) and benzoyl chloride (0.06 mL, 0.86 mmol) was dissolved in CH_2Cl_2 (5 mL) and 10% aqueous NaOH (0.01 mL) was added dropwise under N_2 atmosphere. After 3h stirring at rt, the reaction mixture was added water, extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$ and concentrated. The residue was chromatographed on silica gel to give a white solid (0.12 g, 78%). R_f 0.68 (EtOAc:Hexane = 1:2); mp 60-63 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (2H, d, J = 8.1 Hz), 7.80 (1H, d, J = 8.2 Hz), 7.79 (2H, d, J = 7.8 Hz), 7.63-7.55 (3H, m), 7.51-7.28 (6H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.2, 165.0, 155.7, 137.8, 137.6, 137.1, 134.7, 133.2, 132.6, 132.0, 130.5, 130.6, 129.9, 128.4, 125.6, 121.0; LC-MS (ESI) *m/z* 303.12 [$(M+H)^+$].

2-(4-Chlorobenzoyloxy)-4',5-dichlorobenzophenone (1b): Yield (86%). R_f 0.59 (EtOAc:Hexane = 1:2); mp 124-126 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10-8.00 (4H, m), 7.85 (2H, d, J = 8.0 Hz), 7.71 (1H, d, J = 1.9 Hz), 7.60-7.40 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.9, 160.9, 152.7, 141.8, 139.5, 137.0, 135.9, 134.1, 133.5, 131.1, 131.0, 130.6, 130.5, 128.5, 126.7, 121.5; LC-MS (ESI) *m/z* 404.98 [$(M+H)^+$].

2-(4-Bromobenzoyloxy)-4',5-dibromobenzophenone (1c): Yield (80%). R_f 0.28 (EtOAc:Hexane = 1:2); mp 101-103 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (1H, br s), 7.94 (3H, m), 7.68-7.50 (6H, m), 7.23 (1H, d, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.7, 163.7, 150.4, 139.8, 135.6, 133.8, 133.7, 132.4, 132.0, 131.1, 129.6, 129.0, 128.9, 126.8, 123.3,

119.8; LC-MS (ESI) *m/z* 538.92 [(M+H)$^+$].

2-(4-Iodobenzoyloxy)-4',5-diiodobenzophenone (1d): Yield (86%). R_f 0.30 (EtOAc:Hexane = 1:2); mp 162-164 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (1H, br s), 8.05-7.82 (7H, m), 7.49 (2H, d, *J* = 8.1 Hz), 7.20 (1H, d, *J* = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.9, 161.7, 152.8, 141.8, 140.9, 139.9, 135.1, 132.1, 131.6, 130.2, 130.0, 129.5, 123.6, 101.2, 98.6, 93.9; LC-MS (ESI) *m/z* 680.80 [(M+H)$^+$].

2-(4-Methoxybenzoyloxy)-4',5-dimethoxybenzophenone (1e): Yield (83%). R_f 0.74 (EtOAc:Hexane = 1:2); mp 101-103 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (2H, d, *J* = 8.8 Hz), 7.81-7.01 (9H, m), 3.84 (6H, s), 3.81 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.6, 169.8, 167.2, 163.5, 159.7, 150.3, 140.8, 132.1, 130.1, 129.4, 128.0, 126.6, 123.3, 117.2, 113.4, 108.1, 53.8, 53.8, 53.6; LC-MS (ESI) *m/z* 393.25 [(M+H)$^+$].

2-(4-Benzyloxybenzoyloxy)-4',5-dibenzyloxybenzophenone (1f): Yield (56%). R_f 0.83 (EtOAc:Hexane = 1:4); mp 154-157 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11-7.09 (26H, m), 5.17 (2H, s), 5.16 (2H, s), 5.08 (2H, s); LC-MS (ESI) *m/z* 621.20 [(M+H)$^+$].

2-Benzoyloxy-4',5-dibromobenzophenone (1g). Yield (72%). R_f 0.25 (EtOAc:Hexane = 1:2); mp 106-108 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (2H, d, *J* = 8.3 Hz), 8.01 (1H, br s), 7.98-7.37 (9H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.9, 167.5, 155.0, 137.8, 136.7, 134.3, 134.2, 134.1, 131.5, 130.3, 129.8, 128.6, 126.8, 125.2, 120.9, 118.6; LC-MS (ESI) *m/z* 460.92 [(M+H)$^+$].

2-(4-Bromobenzoyloxy)-4'-bromobenzophenone (1h): Yield (72%). R_f 0.86 (EtOAc:Hexane = 1:2); mp 72-74 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99-7.71 (9H, m), 7.45 (2H, d, *J* = 8.2 Hz), 7.33 (1H, t, *J* = 8.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.7, 162.5, 158.0, 138.4, 132.8, 132.5, 131.1, 130.5, 129.6, 128.2, 128.0, 127.8, 127.0, 126.6, 125.7, 116.9; LC-MS (ESI) *m/z* 460.92 [(M+H)$^+$].

2-Benzoyloxy-5-bromobenzophenone (1i): Yield (82%). R_f 0.87 (EtOAc:Hexane = 1:2); mp 86-89 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (2H, d, *J* = 8.3 Hz), 8.01 (1H, br s), 7.99-7.47 (9H, m), 7.33 (1H, d, *J* = 8.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.9, 160.2, 159.0, 139.1, 138.2, 138.0, 132.9, 132.2, 131.8, 130.4, 129.3, 128.7, 127.6, 123.9, 120.6, 119.9; LC-MS (ESI) *m/z* 381.10 [(M+H)$^+$].

2-(4-Bromobenzoyloxy) benzophenone (1j): Yield (92%). R_f 0.83 (EtOAc:Hexane = 1:2); mp 100-103 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92-7.37 (13H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.6, 166.9, 157.2, 140.7, 132.6, 131.7, 131.0, 130.6, 130.5, 129.1, 128.7, 126.3, 125.9, 124.7, 122.6, 120.1; LC-MS (ESI) *m/z* 381.08 [(M+H)$^+$].

2-(4-Bromobenzoyloxy)-5-bromobenzophenone (1k): Yield (82%). R_f 0.87 (EtOAc:Hexane = 1:2); mp 86-89 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (1H, br s), 7.96-7.47 (10H, m), 7.33 (1H, d, *J* = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.9, 161.2, 155.0, 139.1, 138.2, 138.0, 132.9, 132.2, 131.8, 131.5, 130.4, 129.3, 128.7, 127.6, 120.6, 119.9; LC-MS (ESI) *m/z* 460.98 [(M+H)$^+$].

2-Benzoyloxy-4'-bromobenzophenone (1l): Yield (75%). R_f 0.87 (EtOAc:Hexane = 1:2); mp 76-78 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (2H, d, *J* = 8.4 Hz), 7.90-7.42 (11H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.7, 165.5, 153.1, 139.3, 138.7, 138.0, 135.9, 134.1, 132.2, 131.8, 130.3, 129.5, 128.7, 128.6, 121.6, 118.0; LC-MS (ESI) *m/z* 460.91 [(M+H)$^+$].

Cell Culture and Cell Viability Assay. The immortalized human keratinocyte cell line, HaCaT, was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin G, 100 μg/mL streptomycin) at 37 °C in a humidified incubator containing 5% CO_2 and 95% air. Cell viability was evaluated with a colorimetric assay using MTT [3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide] (Duchefa, Haarlem, Netherlands).[8]

Measurement of IFN-γ-induced ICAM-1 Expression by Western Blot Analysis. Cell lysates were prepared from HaCaT cells. Concentration of proteins in cell lysates was determined by the Bradford protein assay (Bio-Rad, Hercules, CA, USA). Samples of thirty microgram proteins were separated by electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel and were transferred to a nitrocellulose membrane by electroblotting. The membranes were blocked with 10% dry milk in PBS and then incubated with polyclonal rabbit anti-human ICAM-1 (1:1000), or polyclonal rabbit anti-human actin (1:500) antibodies (Santa Cruz, CA, USA). The membranes were then incubated with a goat anti-rabbit IgG antibody (1:750) conjugated to horseradish peroxidase (Sigma, St. Louis, MO, USA). Immunoreactive bands were detected by an enhanced chemiluminescence (ECL) detection kit (Amersham Life Sciences).[9]

RT-PCR Analysis. Total RNA was extracted from HaCaT cells using Trizol reagent kit (Invitrogen, Gaithersburg, MD, USA). RT-PCR was performed as previously described.[9] Two micrograms of total RNA were reverse-transcribed using 10,000 U of reverse transcriptase and 0.5 μg/μL oligo-$(dT)_{15}$ primer (Promega, Madison, WI, USA). cDNA aliquots were subjected to a standard PCR reaction with the following sense and antisense primers (5'→3'): ICAM-1 sense, GGT GAC GCT GAA TGG GGT TCC; ICAM-1 antisense, GTC CTC ATG GTG GGG CTA TGA CTC; β-actin sense, GCG GGA AAT CGT GCG TGA CAT T; and β-actin antisense, GAT GGA GTT GAA GGT AGT TTC GTG. PCR products were resolved on a 1% agarose gel and visualized with UV light after staining with ethidium bromide.

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