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The Role of the Hydrophobic Group on Ring A of Chalcones in the Inhibition of Interleukin-5

Hyun-Mo Yang, Hye-Rim Shin, Soo-Hyun Cho, Gyu-Yong Song, In-Jeong Lee[1], Mi-Kyeong Kim[2], Seung-Ho Lee[3], Jae-Chun Ryu[4], Youngsoo Kim[1], and Sang-Hun Jung*

College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea, [1]College of Pharmacy and Research Center for Bioresource and Health, Chungbuk National University, Cheongju 361-763, Korea, [2]College of Medicine, Chungbuk National University, Cheongju 361-763, Korea, [3]College of Pharmacy, Yeungnam University, Kyungsan 712-749, Korea, and [4]Toxicology Laboratory, Korea Institute of Science and Technology, Seoul 130-650, Korea

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Novel chalcones were found as potent inhibitors of interleukin-5 (Il-5). 1-(6-Benzyloxy-2-hydroxyphenyl)-3-(4-hydroxyphenyl)propenone (**2a**, 78.8 % inhibition at 50 μM, IC_{50} = 25.3 μM) was initially identified as a potent inhibitor of IL-5. This activity is comparable to that of budesonide or sophoricoside (**1a**). The benzyloxy group appears to be critical for the enhancement of the IL-5 inhibitory activity. To identify the role of this hydrophobic moiety, cyclohexyloxy (**2d**), cyclohexylmethoxy (**2c**), cyclohexylethoxy (**2e**), cyclohexylpropoxy (**2f**), 2-methylpropoxy (**2g**), 3-methylbutoxy (**2h**), 4-methylpentoxy (**2i**), and 2-ethylbutoxy (**2j**) analogs were prepared and tested for their effects on IL-5 bioactivity. Compounds **2c** (IC_{50} = 12.6 μM), **2d** (IC_{50} = 12.2 μM), and **2i** (IC_{50} = 12.3 μM) exhibited the most potent activity. Considering the cLog P values of **2**, the alkoxy group contributes to the cell permeability of **2** for the enhancement of activity, rather than playing a role in ligand motif binding to the receptor. The optimum alkoxy group in ring A of **2** should be one that provides the cLog P of **2** in the range of 4.22 to 4.67.

Key words: Chalcones, Inhibitor, Interleukin-5

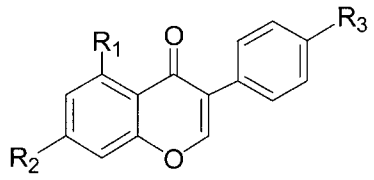
INTRODUCTION

Eosinophilic inflammation is the main histological correlate of airway hyperresponsiveness and tissue injury in the pathogenesis of bronchial asthma (Djukanovic, 2000; Kraneveld *et al.*, 1997). There is strong evidence for a central role of T helper type 2 (Th2) cytokines, which contribute significantly to diseases such as asthma, and therapeutic strategies that target Th2 cytokines are of potential benefit in allergic disease (Gelfand, 1998; Riffo-Vasquez and Spina, 2002). Interleukin-5 (IL-5) appears to be one of the main proinflammatory mediators among a growing number of cytokines and chemokines that induce eosinophilic inflammation (Hamelmann *et al.*, 1999; Allakhverdi *et al.*, 2002). IL-5 displays its cellular response by binding to a specific receptor (IL-5R) composed of two distinct polypeptides of α and β chains (Takaki *et al.*, 2000). Alone, the IL-5Rα binds to IL-5 with low affinity (Murata *et al.*, 1992). Even though IL-5Rβ alone does not bind to IL-5, it is required for high affinity binding in combination with IL-5Rα and is essential for signal transduction (Mita *et al.*, 1993). IL-5Rβ is also shared by IL-3 and GM-CSF receptors as the common signal transducer, and thereby these cytokines display several overlapping biological effects (Tomaki *et al.*, 2002; Bagley *et al.*, 2001). Rather than coordinating T-cell effector development or antibody isotype secretion by B-cells, the biological effects of IL-5 are confined primarily to growth, differentiation, survival, chemotaxis, and activation of eosinophils (Mishra *et al.*, 2002; Webb *et al.*, 2000; Lee *et al.*, 1997). The contribution of IL-5 to allergic disease has been greatly demonstrated by the generation of mice deficient in the IL-5 gene. In contrast to normal mice, allergic IL-5-deficient mice do not generate eosinophilia in the bone marrow, blood or lung in response to allergen provocation (Hogan *et al.*, 1998). Airway instillation of recombinant IL-5 to allergic IL-5-deficient mice completely restores allergen-induced eosinophilia to levels normally observed in allergic wild-type mice (Foster *et al.*, 1996).

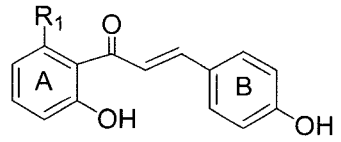
Correspondence to: Sang-Hun Jung, College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
Tel. 82-42-821-5939, Fax. 82-42-823-6566
E-mail: jungshh@cnu.ac.kr

Thus, IL-5 is critically involved in eosinophilia-associated allergic inflammation.

Interfering with the action of IL-5 represents one of the new immunomodulatory therapeutic strategies in the treatment of allergic diseases, including bronchial asthma. Compared to established immunosuppressive agents like corticosteroids, a major advantage of this strategy is the specificity of reducing eosinophilic inflammation, thus providing possible benefits without other side effects. However, small organic compounds to inhibit IL-5 activity have rarely been found. The isothiazolones were identified as IL-5 antagonists through modification of Cys66 in IL-5R (Devos *et al.*, 1994) Sophoricoside (**1a**) and its analogs (Fig. 1) were isolated from *Sophora japonica*, a plant in the Leguminosae family, as inhibitors of IL-5 bioactivity (Min *et al.*, 1999) and showed differential inhibition of IL-3 and GM-CSF bioactivities (Yun *et al.*, 2000). During the study of structure activity relationships of sophoricoside analogs (Jung *et al.*, 2003), chalcones **2a** and **2b** were prepared as intermediates for the preparation of the corresponding isoflavonones, and their inhibitory effects on IL-5 bioactivity have been evaluated as shown in Table I. The inhibitory activity of (*E*)-1-(6-benzyloxy-2-hydroxyphenyl)-3-(4-hydroxyphenyl)-2-propen-1-one (**2a**, 78.8% inhibition at 50 μM, IC_{50} = 25.3 μM) is comparable to the most potent 5-benzyloxy-3-(4-hydroxyphenyl)chromen-4-one (**1b**, 87.9% inhibition at 50 μM, IC_{50} = 15.3 μM) (Jung *et al.*, 2003). However, deletion of benzyloxy group, as shown in **2b** (50.0% inhibition at 50 M, IC_{50} = 48.2 μM), reduced the activity considerably. The saturation of benzyloxy to cyclohexylmethoxy increases the activity as shown in the activity of **2c** (99.0% inhibition at 50 μM, IC_{50} = 12.6 μM). Thus, the hydrophobic groups at 2-position of chalcones **2** were considered to be very important for the enhancement of the IL-5 inhibition. Therefore, chalcones **2** substituted with various alkoxy groups were investigated to determine the role of the hydrophobic group.



1a: Sophoricoside R_1, R_2 = OH, R_3= OGlu
1b: R_1 = benzyloxy, R_2 = H, R_3 = OH
1c: R_1 = H, R_2 = benzyloxy, R_3 = OH



2: R_1, substituents listed in Table 1

Fig. 1. Isoflavonoids (**1**) and Chalcones (**2**)

MATERIALS AND METHODS

Melting points (mp) were determined on the Electrothermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained, and all solvents were purified by the standard procedures prior to use (Perrin *et al.*, 1982). Thin-layer chromatography was performed on Merck silica gel GF-254 precoated plates and the identification was done with UV light and colorization with 10% phosphomolybdic acid spray followed by

Table 1. Inhibitory activity of chalcone analogs 2 against IL-5

Entry No.	Compound No.	Substituents	% Inhibition at 50 μM[a)]	IC_{50} (μM)[a)]	D (Å)[b)]	cLog P[c)]
1	**2a**	benzyloxy	78.8 ± 1.4	25.3		4.23
2	**2b**	H	50.0 ± 2.2	48.2		2.81
3	**2c**	cyclohexylmethoxy	99.0 ± 0.5	12.6	6.288	4.66
4	**2d**	cyclohexyloxy	91.5 ± 0.5	12.2	5.403	4.23
5	**2e**	cyclohexylethoxy	71.8 ± 4.2	29.8	6.756	5.00
6	**2f**	cyclohexylpropoxy	91.3 ± 4.6	27.2	8.832	5.42
7	**2g**	2-methylpropoxy	76.6 ± 5.3	23.2	4.641	3.90
8	**2h**	3-methylbutoxy	78.0 ± 1.0	16.8	6.427	4.25
9	**2i**	4-methylpentoxy	99.0 ± 0.5	12.6	7.702	4.67
10	**2j**	2-ethylbutoxy	82.5 ± 4.5	16.1	5.811	4.74
11	Budesonide		70.3 ± 2.1	26.8		

[a)]Data are mean ± SD obtained from triplicate experiments. [b)]D; distance from oxygen to terminal carbon of stretched conformation of alkoxy group. [c)] cLog P was calculated based on Crippen's fragmentation (Ghose *et al.*, 1987).

heating. Flash column chromatography was performed with E. Merck silica gel (230-400 mesh). IR spectra were recorded with the Jasco IR-Report-100 IR spectrometer in cm^{-1} and corrected against a polystyrene peak at 1601 cm^{-1}. NMR spectra were measured against the tetramethylsilane peak using the Varian Unity Inova 400NMR (400 MHz) spectrometers. Elemental analysis was performed with the EA1110 elemental analyzer (CE Instrument).

General procedure for the preparation of compounds 4 (Jung *et al.*, 2003)

A mixture of 2,6-dihydroxyacetophenone (40 mmol) and potassium carbonate (48.0 mmol) in dry acetonitrile (80 mL) was refluxed for two hours. The solution of 18-crown-6 ether (0.1 equivalents) and the appropriate alkyl methanesulfonate (48.0 mmol) in acetonitrile (10 mL) was added and then the reaction mixture was refluxed for one day. After cooling to room temperature, dichloromethane (320 mL) was added and the mixture was washed with water three times. The organic layer was dried with anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by flash column chromatography.

6-Cyclohexylmethoxy-2-hydroxyacetophenone (4c)

Cyclohexylmethyl methanesulfonate was used as the alkyl methanesulfonate. Yield 71.0%; yellow solid; mp 71.3~72.4 °C; Rf 0.30 (hexanes : ethyl acetate = 20 : 1); IR (KBr) 3420, 3050, 2900, 1620 cm^{-1}; NMR ($CDCl_3$) δ 1.08~1.89 (m, 11H), 2.71 (s, 3H), 3.83 (d, J = 5.6 Hz, 2H), 6.36 (d, J = 8.4 Hz, 1H), 6.54 (d, J = 8.4 Hz, 1H), 7.31 (t, J = 8.4 Hz, 1H), 13.20 (s, 1H).

6-(Cyclohexyloxy)-2-hydroxyacetophenone (4d)

Cyclohexyl methanesulfonate was used as the alkyl methanesulfonate. Yield 72.0%; white solid; Rf 0.36 (hexanes : ethyl acetate = 18 : 1); IR (KBr) 3450, 2900, 1620 cm^{-1}; NMR ($CDCl_3$) δ 1.23~2.09 (m, 10H), 2.73 (s, 3H), 4.40 (m, 1H), 6.37 (d, J = 8.4 Hz, 1H), 6.49 (d, J = 8.4 Hz, 1H), 7.29 (t, J = 8.4 Hz, 1H), 13.21 (s, 1H).

6-(2-Cyclohexylethoxy)-2-hydroxyacetophenone (4e)

2-Cyclohexylethyl methanesulfonate was used as the alkyl methanesulfonate. Yield 60.0%; yellow solid; mp 68.9-69.7 °C; Rf 0.29 (hexanes : ethyl acetate = 20 : 1); IR (KBr) 3450, 2930, 2850, 1620 cm^{-1}; NMR ($CDCl_3$) δ 1.10~2.10 (m, 13H), 2.68 (s, 3H), 4.07 (t, J = 6.6 Hz, 2H), 6.36 (d, J = 8.4 Hz, 1H), 6.53 (d, J = 8.4 Hz, 1H), 7.31 (t, J = 8.3 Hz, 1H), 13.20 (s, 1H).

6-(3-Cyclohexylpropoxy)-2-hydroxyacetophenone (4f)

3-Cyclohexylpropyl methanesulfonate was used as the alkyl methanesulfonate. Yield 93.2%; white solid; mp 71.4-72.3 °C; Rf 0.33 (hexanes : ethyl acetate = 18 : 1); IR (KBr) 3450, 2900, 1650 cm^{-1}; NMR (acetone-d_6) δ 0.87~1.84 (m, 15H), 2.69 (s, 3H), 4.12 (t, J = 6.4 Hz, 2H), 6.43 (d, J = 8.4 Hz, 1H), 6.52 (d, J = 8.4 Hz, 1H), 7.39 (t, J = 8.3 Hz, 1H), 13.10 (s, 1H).

6-(2-Methylpropoxy) -2-hydroxyacetophenone (4g)

For the preparation of **4g**, 2-methyl-1-bromopropane was used as the alkylating agent. Yield 48.5%; yellow oil; Rf 0.31 (hexanes : ethyl acetate = 18 : 1); IR (film) 3450, 2950, 1620 cm^{-1}; NMR (acetone-d_6) δ 1.08 (d, J = 6.4 Hz, 6H), 2.19 (m, 1H), 2.71 (s, 3H), 3.91 (t, J = 6.4 Hz, 2H), 6.47 (d, J = 8.4 Hz, 1H), 6.55 (d, J = 8.4 Hz, 1H), 7.30 (t, J = 8.4 Hz, 1H), 13.25 (s, 1H).

6-(3-Methylbutoxy)-2-hydroxyacetophenone (4h)

3-Methylbutyl methanesulfonate was used as the alkyl methanesulfonate. Yield 72.3%; white solid, Rf 0.32 (hexanes : ethyl acetate = 18 : 1); IR (KBr) 3450, 2930, 2850, 1620 cm^{-1}; NMR ($CDCl_3$) δ 0.98 (d, J = 6.8 Hz, 6H), 1.75 (m, 2H), 1.85 (m, 1H), 2.68 (s, 3H), 4.05 (t, J = 6.4 Hz, 2H,) 6.36 (d, J = 8.4 Hz, 1H), 6.53 (d, J = 8.4 Hz, 1H), 7.30 (t, J = 8.4 Hz, 1H), 13.25 (s, 1H).

2-(4-Methylpentyloxy)-6-hydroxyacetophenone (4i)

4-Methylpentyl methanesulfonate was used as the alkyl methanesulfonate. Yield 71.2%; white solid; Rf 0.33 (hexanes : ethyl acetate = 18 : 1); IR (KBr) 3450, 2930, 2850, 1620 cm^{-1}; NMR ($CDCl_3$) δ 0.93 (d, J = 6.4 Hz, 6H), 1.36(m, 2H), 1.62 (m, 1H), 1.85 (m, 2H), 2.68 (s, 3H), 4.01 (t, J = 6.4 Hz, 2H), 6.34 (d, J = 8.4Hz, 1H), 6.52 (d, J = 8.4Hz, 1H), 7.29 (t, J = 8.4 Hz, 1H), 13.26 (s, 1H).

2-(2-Ethylbutoxy)-6-hydroxyacetophenone (4j)

2-Ethylbutyl methanesulfonate was used as the alkyl methanesulfonate. Yield 70.7%; white solid; Rf 0.25 (hexane : ethyl acetate = 17 : 1); IR (KBr) 3450, 2900, 1620 cm^{-1}; NMR ($CDCl_3$) δ 0.95 (t, J = 7.6 Hz, 6H), 1.51 (m, 4H), 1.73 (m, 1H), 2.69 (s, 3H), 3.94 (d, J = 6.4 Hz, 2H), 6.39 (d, J = 8.4 Hz, 1H), 6.53 (d, J = 8.4 Hz, 1H), 7.31 (t, J = 8.8 Hz, 1H), 13.30 (s, 1H).

General procedure for the preparation of compounds 5

The corresponding 2-hydroxyacetophenone **4** (one equivalent) was added to two equivalents of sodium hydroxide or potassium hydroxide in 90 % aqueous ethanol solution (8.7% w/w). The substituted 4-(methoxymethoxy)benzaldehyde (Jung *et al.*, 2003) (1.02 equivalent) was then added, and the resulting solution was stirred for two hours at 50 °C. After removal of ethanol under vacuum, the crude mixture was dissolved

in water and neutralized with hydrochloric acid. The resulting mixture was extracted with dichloromethane three times. The combined organic layers were dried with anhydrous sodium sulfate and evaporated under vacuum. The crude product was purified by flash column chromatography to give **5**.

(*E*)-1-(6-Cyclohexylmethoxy-2-hydroxyphenyl)-3-[4-(methoxymethoxy)phenyl]-2- propen-1-one (5c)
Compound **4c** was used as the 2-hydroxyacetophenone. Yield 71.1%, yellow solid; mp 90.4~91.7 °C; Rf 0.36 (hexanes : ethyl acetate = 7 : 1); IR (KBr) 3350, 3100, 2950, 1630 cm^{-1}; NMR ($CDCl_3$) δ 1.01~1.88 (m, 11H), 3.49 (s, 3H), 3.86 (d, *J* = 5.6 Hz, 2H,), 5.22 (s, 2H), 6.40 (d, *J* = 7.6 Hz, 1H), 6.59 (d, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 8.8 Hz, 2H), 7.32 (t, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.79 (d, *J* = 15.6 Hz, 1H), 7.85 (d, *J* = 15.6 Hz, 1H), 13.25 (s. 1H).

(*E*)-1-(6-Cyclohexyloxy-2-hydroxyphenyl)-3-[4-(methoxymethoxy)phenyl]prop-2-en-1-one (5d)
Compound **4d** was used as the 2-hydroxyacetophenone. Yield 46.5%; yellow solid; mp 50.2 - 51.4 °C; Rf 0.32 (hexanes : ethyl acetate = 7 : 1); IR (KBr) 3450, 2930, 2850, 1625 cm^{-1}; NMR ($CDCl_3$) δ 1.19 (m, 10H), 3.49 (s, 3H), 4.43 (m, 1H), 5.22 (s, 2H), 6.42 (d, *J* = 8.4 Hz, 1H), 6.55 (d, *J* = 8.4 Hz, 1H), 7.06 (d, *J* = 8.4 Hz, 2H), 7.29 (t, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 15.6 Hz, 1H), 7.86 (d, *J* = 15.6 Hz, 1H), 13.25 (s, 1H).

(*E*)-1-[6-(2-Cyclohexylethoxy)-2-hydroxyphenyl]-3-[4-(methoxymethoxy)phenyl]prop-2-en-1-one (5e)
Compound **4e** was used as the 2-hydroxyacetophenone. Yield 75.7%; orange solid; Rf 0.35 (hexanes : ethyl acetate = 7 : 1); IR (KBr) 3450, 2930, 2850, 1625 cm^{-1}; NMR (acetone-d_6) δ 0.84~1.20 (m, 5H) 1.50-1.86 (m, 8H), 3.44 (s, 3H), 4.19 (t, *J* = 6.4 Hz, 2H), 5.27 (s, 2H), 6.53 (d, *J* = 8.4 Hz, 1H), 6.61 (d, *J* = 8.4 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 7.39 (t, *J* = 8.4 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 15.6Hz, 1H), 7.90 (d, *J* = 15.6 Hz, 1H), 13.05 (s, 1H).

(*E*)-1-[6-(3-Cyclohexylpropoxy)-2-hydroxyphenyl]-3-[4-(methoxymethoxy)phenyl]prop-2-en-1-one (5f)
Compound **4f** was used as the 2-hydroxyacetophenone. Yield 62.8%; yellow solid; mp 103.2 - 104.6 °C; Rf 0.43 (hexanes : ethyl acetate = 18 : 1), IR (KBr) 3450, 2930, 1625 cm^{-1}; NMR (acetone-d_6) δ 0.78 (m, 2H), 1.06 - 1.19 (m, 4H), 1.37 (m, 2H), 1.62 (m, 5H), 1.93 (m, 2H), 3.46 (s, 3H), 4.15 (t, *J* = 6.0Hz, 2H), 5.27 (s, 2H), 6.53 (d, *J* = 8.4Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 7.40 (t, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 15.6 Hz, 1H), 7.91 (d, *J* = 15.6 Hz, 1H), 13.00 (s, 1H).

(*E*)-1-[6-(2-Methylpropoxy)-2-hydroxyphenyl]-3-[4-(methoxymethoxy)phenyl]prop-2-en-1-one (5g)
Compound **4g** was used as the 2-hydroxyacetophenone. Yield 73.1%; Orange oil; Rf 0.32 (hexanes : ethyl acetate = 7 : 1); IR (film) 3450, 2950, 1635 cm^{-1}; NMR (acetone-d_6) δ 1.05 (d, *J* = 6.8 Hz, 6H), 2.28 (m, 1H), 3.45 (s, 3H), 3.94 (d, *J* = 2.0 Hz, 2H), 5.27 (s, 2H), 6.53 (d, *J* = 8.4 Hz, 1H), 6.57(d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 2H), 7.39 (t, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 15.6 Hz, 1H), 7.89 (d, *J* = 15.6 Hz, 1H), 12.95 (s, 1H).

(*E*)-1-[6-(3-Methylbutoxy)-2-hydroxyphenyl]-3-[4-(methoxymethoxy)phenyl]prop-2-en-1-one (5h)
Compound **4h** was used as the 2-hydroxyacetophenone. Yield 49.5%; yellow oil; Rf 0.35 (hexanes : ethyl acetate = 7 : 1); IR (KBr) 3450, 2930, 2850, 1625 cm^{-1}; NMR ($CDCl_3$) δ 0.92 (d, *J* = 6.8 Hz, 6H), 1.77 (q, *J* = 6.4 Hz, 2H), 1.85 (m, 1H), 3.51 (s, 3H), 4.09 (t, *J* = 6.4 Hz, 2H), 6.41 (d, *J* = 8.4 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 7.05 (d, *J* = 8.8 Hz, 2H), 7.31 (t, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.80 (d, *J* = 15.6 Hz, 1H), 7.84 (d, *J* = 15.6 Hz, 1H), 13.25 (s, 1H).

(*E*)-1-[6-(4-Methylpentoxy)-2-hydroxyphenyl]-3-[4-(methoxymethoxy)phenyl] prop-2-en-1-one (5i)
Compound **4i** was used as the 2-hydroxyacetophenone. Yield 35.0%; yellow oil; Rf 0.37 (hexanes : ethyl acetate = 7 : 1); IR (KBr) 3450, 2930, 2850, 1625 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (d, *J* = 6.8 Hz, 6H), 1.35 (m, 2H), 1.54 (m, 1H), 1.88 (m, 2H), 3.48 (s, 3H), 4.05 (t, *J* = 6.4 Hz, 2H), 5.21 (s, 2H), 6.39 (d, *J* = 8.4 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 7.05 (d, *J* = 8.8 Hz, 2H), 7.32 (t, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 15.2 Hz, 1H), 7.85 (d, *J* = 15.6 Hz, 1H), 13.21 (s, 1H).

(E)-1-[6-(2-Ethylbutoxy)-2-hydroxyphenyl]-3-[4-(methoxymethoxy)phenyl]prop-2-en-1-one (5j)
Compound **4j** was used as the 2-hydroxyacetophenone. Yield 45.2 %; yellow oil; Rf 0.38 (hexanes : ethyl acetate = 7 : 1); IR (KBr) 3450, 2930, 2850, 1625 cm^{-1}; NMR ($CDCl_3$) δ 0.89 (t, *J* = 7.6 Hz, 6H), 1.48 (m, 4H), 1.74 (m, 1H), 3.49 (s, 3H), 3.97 (d, *J* = 5.6 Hz, 2H), 5.22 (s, 2H), 6.43 (d, *J* = 8.4 Hz, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 7.32 (t, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.79 (m, 2H), 13.14 (s, 1H).

General procedure for the preparation of compounds 2

The corresponding methoxymethoxy chalcones **5** (0.50 g) was dissolved in methanol (20 mL), and hydrochloric acid (2 *N*, 2 mL) was added. The resulting solution was stirred at 40 °C for 6 h. After removal of solvent under vacuum, the crude mixture was dissolved in dichloromethane and washed with water three times. The organic

layer was dried with anhydrous sodium sulfate and evaporated under vacuum. The crude product was purified by flash column chromatography to give **2**.

(*E*)-1-(6-Benzyloxy-2-hydroxyphenyl)-3-(4-hydroxy-phenyl)prop-2-en-1-one (2a)

(*E*)-1-(2-Benzyloxy-6-hydroxyphenyl)-3-(4-methoxymethoxy-phenyl)-2-propen-1-one (Jung *et al.*, 2003) was used for the preparation of **2a**. Yield 86.4%; orange solid; mp 143.2~144.3 °C; Rf 0.26 (hexanes : ethyl acetate = 3 : 1); IR (KBr) 3400, 3100, 2950, 1640 cm^{-1}; NMR ($CDCl_3$) δ 5.19 (s, 2H), 6.69~6.89 (m, 3H), 7.25 (d, *J* = 8.6 Hz, 1H), 7.38 (d, *J* = 8.8 Hz, 2H), 7.50 (m, 6H), 7.76 (d, *J* = 15.6 Hz, 2H), 13.64 (s, 1H); Theoretical elemental analysis calculated for $C_{22}H_{18}O_4$: C 76.29, H 5.24; Experimental values: C 76.18, H 5.15.

(*E*)-3-(4-Hydroxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (2b)

(*E*)-1-(2-hydroxyphenyl)-3-(4-methoxymethoxyphenyl) prop-2-en-1-one (Jung *et al.*, 2003) was used for the preparation of **2b**. Yield 93.8%; orange solid; mp 133.5~134.7 °C; Rf 0.20 (hexanes : ethyl acetate = 3 : 1); IR (KBr) 3300, 3100, 2950, 1640 cm^{-1}; NMR ($CDCl_3$) δ 6.90 (d, *J* = 8.4 Hz, 1H), 6.98 (m, 2H), 7.50 (t, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 15.6 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.89 (d, *J* = 15.6 Hz, 1H), 7.92 (d, *J* = 6.4 Hz, 2H), 12.92 (s, 1H); Theoretical elemental analysis calculated for $C_{15}H_{12}O_3$: C 74.99, H 5.03; Experimental values: C 74.73, H 4.97.

(*E*)-1-(6-Cyclohexylmethoxy-2-hydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (2c)

Compound **5c** was used for the preparation of **2c**. Yield 52.9%; yellow solid; mp 173.7~174.4°C; Rf 0.31 (hexanes : ethyl acetate = 3 : 1); IR (KBr) 3400, 3100, 2850, 1620 cm^{-1}; NMR ($CDCl_3$) 1.06~1.71 (m, 11H), δ 3.86 (d, *J* = 6.4, 2H), 6.40 (d, *J* = 8.4 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 2H), 7.32 (t, *J* = 8.4 Hz,1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 15.6 Hz, 1H), 7.84 (d, *J* = 15.6 Hz, 1H), 13.27 (s. 1H); Theoretical elemental analysis calculated for $C_{22}H_{24}O_4$: C 74.98, H 6.86; Experimental values: C 74.79, H 6.77.

(*E*)-1-(6-Cyclohexyloxy-2-hydroxyphenyl)-3-(4-hydroxy-phenyl)prop-2-en-1-one (2d)

Compound **5d** was used for the preparation of **2d**. Yield 82%; yellow oil; Rf 0.21 (hexanes : ethyl acetate = 3 : 1); IR (film) 3350, 2930, 2850, 1620 cm^{-1}; NMR ($CDCl_3$) δ 1.25-1.43 (m, 4H),1.54-1.67 (m, 2H), 1.76-1.79 (m, 2H), 2.01-2.06 (m, 2H) 4.43 (m, 1H), 6.13 (br s, 1H), 6.42 (d, *J* = 8.4 Hz, 1H), 6.55 (d, *J* = 8.4 Hz, 1H,), 6.87 (d, *J* = 8.8 Hz, 2H), 7.29 (t, *J* = 8.4 Hz, 1H), 7.75 (d, *J* = 15.6 Hz, 1H), 7.83 (d, *J* = 15.6 Hz, 1H), 13.05 (s, 1H); Theoretical elemental analysis calculated for $C_{21}H_{22}O_4$: C 74.54, H 6.55; Experimental values: C 74.38, H 6.47.

(*E*)-1-[6-(2-Cyclohexylethoxy)-2-hydroxyphenyl]-3-(4-hydroxy-phenyl)prop-2-en-1-one (2e)

Compound **5e** was used for the preparation of **2e**. Yield 78.8%; yellow solid; mp 161.2-162.4 °C; Rf 0.24 (hexanes : ethyl acetate = 3 : 1); IR (KBr) 3350, 2930, 2850, 1620 cm^{-1}; NMR (acetone-d_6) δ 0.86 -1.84 (m, 13H), 4.19 (t, *J* = 6.4 Hz, 2H), 6.52 (d, *J* = 8.4 Hz, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 7.38 (t, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 15.6 Hz, 1H), 7.88 (d, *J* = 15.6 Hz, 1H), 9.00 (br s, 1H), 13.14 (s, 1H); Theoretical elemental analysis calculated for $C_{23}H_{26}O_4$: C 75.38, H 7.15; Experimental values: C 75.16, H 7.02.

(*E*)-1-[6-(3-Cyclohexylpropoxy)-2-hydroxyphenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one (2f)

Compound **5f** was used for the preparation of **2f**. Yield 82.2 %; orange solid; mp 120.3-121.2 °C; Rf 0.25 (hexanes : ethyl acetate = 3 : 1); IR (KBr) 3400, 2930, 1620 cm^{-1}; NMR (acetone-d_6) 0.72-0.84 (m, 2H), 1.07-1.28 (m, 5H), 1.33-1.39 (m, 2H), 1.57-1.64 (m, 4H) 1.87-1.94 (m, 2H), 4.12 (t, *J* = 6.4 Hz, 2H), 6.52 (d, *J* = 8.4 Hz, 1H), 6.55 (d, *J* = 8.4 Hz, 1H), 6.94 (d, *J* = 8.8 Hz, 2H), 7.38 (t, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 15.6 Hz, 1H), 7.86 (d, *J* = 15.6 Hz, 1H), 9.02 (br s, 1H), 13.10 (s, 1H); Theoretical elemental analysis calculated for $C_{24}H_{28}O_4$: C 75.76, H 7.42; Experimental values: C 75.57, H 7.32.

(*E*)-1-[6-(2-methylpropoxy)-2-hydroxyphenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one (2g)

Compound **5g** was used for the preparation of **2g**. Yield 82.1%; yellow solid; mp 136.6-137.2 °C; Rf 0.21 (hexanes : ethyl acetate = 3 : 1); IR (KBr) 3250, 2950, 1620 cm^{-1}; NMR (acetone-d_6) δ 1.05 (d, *J* = 6.8 Hz, 6H), 2.26 (m, 1H), 3.94 (d, *J* = 6.4 Hz, 2H), 6.52 (d, *J* = 8.4 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 7.39 (t, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 15.6 Hz, 1H), 7.87 (d, *J* = 15.6 Hz, 1H), 9.00 (br s, 1H), 12.97 (s, 1H); Theoretical elemental analysis calculated for $C_{19}H_{20}O_4$: C 73.06, H 6.45; Experimental values: C 72.87, H 6.32.

(*E*)-1-[6-(3-methylbutoxy)-2-hydroxyphenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one (2h)

Compound **5h** was used for the preparation of **2h**. Yield 78.5 %; yellow solid; mp 78.2-79.1 °C; Rf 0.23 (hexanes : ethyl acetate = 3 : 1); IR (KBr) 3350, 2930, 2850, 1620 cm^{-1}; NMR ($CDCl_3$) δ 0.91 (d, *J* = 6.4 Hz, 6H), 1.76 (q, *J* = 6.4 Hz, 2H), 1.85 (m, 1H), 4.09 (t, *J* = 6.4 Hz, 2H), 6.01 (br s, 1H), 6.42 (d, *J* = 8.4 Hz, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 2H), 7.32 (t, *J* = 8.4 Hz, 1H), 7.49

(d, J = 8.8 Hz, 2H), 7.76 (d, J = 15.6 Hz, 1H), 7.83 (d, J = 15.6 Hz, 1H), 13.37 (s, 1H); Theoretical elemental analysis calculated for $C_{20}H_{22}O_4$: C 73.60, H 6.79; Experimental values: C 73.47, H 6.57.

(*E*)-1-[6-(3-Methylpentoxy)-2-hydroxyphenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one (2i)

Compound **5i** was used for the preparation of **2i**. Yield 81.2%; yellow solid; mp 130.2-131.4 °C; Rf = 0.24 (hexanes : ethyl acetate = 3 : 1); IR (KBr) 3350, 2930, 2850, 1620 cm^{-1}; NMR ($CDCl_3$) δ 0.84 (d, J = 6.8 Hz, 6H), 1.35 (m, 2H), 1.54 (m, 1H), 1.88 (m, 2H), 4.05 (t, J = 6.8 Hz, 2H), 5.50 (br s, 1H), 6.39 (d, J = 8.4 Hz, 1H), 6.58 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 8.8 Hz, 2H), 7.32 (t, J = 8.4 Hz, 1H), 7.52 (d, J = 8.8 Hz, 2H), 7.77 (d, J = 15.6 Hz, 1H), 7.84 (d, J = 15.6 Hz, 1H), 13.21 (s, 1H); Theoretical elemental analysis calculated for $C_{21}H_{24}O_4$: C 74.09, H 7.11; Experimental values: C 73.89, H 6.98.

(*E*)-1-[6-(2-Ethylbutoxy)-2-hydroxyphenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one (2j)

Compound **5j** was used for the preparation of **2j**. Yield 78.9 %; yellow oil; Rf 0.24 (hexanes : ethyl acetate = 3 : 1); IR (KBr) 3350, 2930, 2850, 1620 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (t, J = 7.6 Hz, 6H), 1.47 (m, 4H), 1.73 (m, 1H), 3.96 (d, J = 5.6 Hz, 2H), 5.80 (br s, 1H), 6.43 (d, J = 8.4 Hz 1H), 6.58 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 8.8 Hz, 2H), 7.31 (t, J = 8.4 Hz, 1H), 7.48 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 15.6, 1H), 13.24 (s, 1H); Theoretical elemental analysis calculated for $C_{21}H_{24}O_4$: C 74.09, H 7.11; Experimental values: C 73.92, H 7.01.

Inhibitory activity against interleukin-5

The Y16 cell line was grown at 37 °C with 5% CO_2 in RPMI (10.4 mg/mL RPMI-1640, 24 mM $NaHCO_3$, 100 units/mL benzylpenicillin potassium, 100 μg/mL streptomycin sulfate, pH 7.1) containing 8% fetal bovine serum (FBS) and 5 units/mL IL-5. The Y16 cells were subsequently harvested by centrifugation at 250 x g for 10 minutes at 4 °C, washed twice with Hanks' solution (9.8 mg/mL Hanks' balanced salts, 24 mM $NaHCO_3$, pH 7.1), and resuspended in a small volume of RPMI containing 8% FBS. The number of cells collected were counted after trypan blue exclusion and then diluted to 1×10^5 cells/mL with RPMI containing 8 % FBS. Viability of the cells was greater than 95 % in all preparations. One hundred L, containing 1×10^4 cells, were dispensed to each well of a 96-well microplate (Nunc, Denmark), and 50 μL of 4 units/mL IL-5 and 50 μL of the test compounds were added. The control group was treated with RPMI containing 8 % FBS instead of a test compound, and the blank group received RPMI containing 8% FBS instead of IL-5. After incubation at 37 °C with 5% CO_2 for 48 hours, Y16 cells in each well were treated with 20 μL of WST-1 solution (3.3 mg WST-1 and 0.7 mg 1-methoxy-5-methyl-phenazinium methylsulfate per mL of phosphate-buffered saline) and incubated at 37 °C with 5% CO_2 for 4 hours. The absorbance at wavelength 450 nm (A_{450}) was then measured using a microplate reader (Molecular Device, USA). The inhibitory effect on the IL-5 bioassay by each test compound at 50 μM concentration was expressed as % inhibition, [1-(sample A_{450}-blank A_{450})/(control A_{450}-blank A_{450})] x 100. Data were collected as mean ± SEM of three independent tests. Test compounds exhibiting greater than 50% inhibition at 50 μM concentration were serially diluted, and then various dilutions were used in the IL-5 bioassay to determine IC_{50} values.

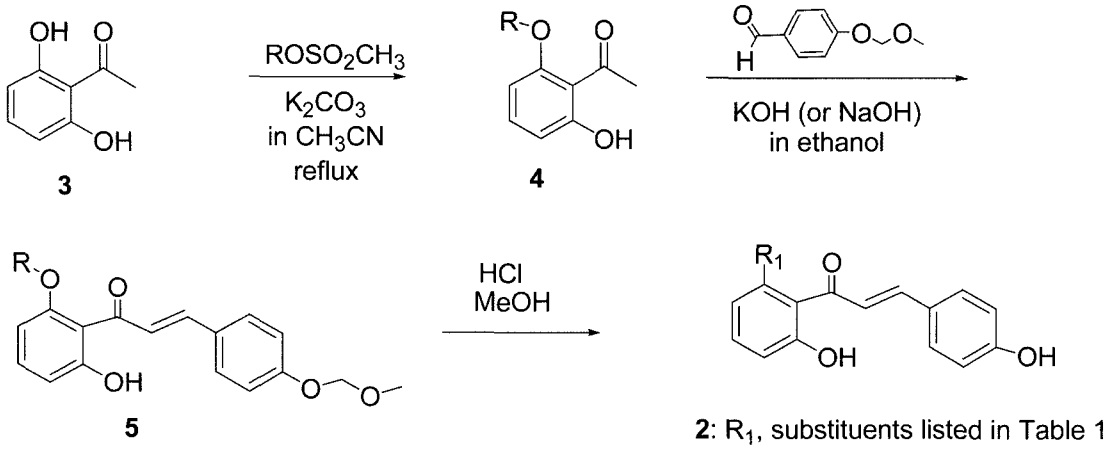
RESULTS AND DISCUSSION

To determine the role of the alkoxy group at the 6-position on ring A of **2** in the inhibition of IL-5 bioactivity, four cyclohexyl analogs and four acyclic analogs were designed as shown in Fig. 1. In the cyclohexyl analogs, the number of methylene units between the oxygen on ring A and the cyclohexyl was adjusted. Thus cyclohexyloxy (**2d**), cyclohexylmethoxy (**2c**), cyclohe-xylethoxy (**2e**), and cyclohexylpropoxy (**2f**) analogs were prepared and tested for inhibition of IL-5 bioactivity. In the acyclic analogs, methyl branched derivatives were designed in order to generate a similar shape to the cyclohexyl analogs. Thus 2-methylpropoxy (**2g**), 3-methylbutoxy (**2h**), 4-methylpentoxy (**2i**), and 2-ethylbutoxy (**2j**) analogs were prepared and tested for inhibition of IL-5 bioactivity.

The preparation of **2** is illustrated in Scheme 1. Aldol condensation of substituted 2-hydroxyacetophenones **4** and 4-methoxymethoxybenzaldehyde in the presence of sodium hydroxide or potassium hydroxide in ethanol at 50 °C for two hours gave compounds **5**. These were then hydrolyzed to generate **2**. Without the protection of 4-hydroxybenzaldehyde, the yield was very low in the aldol condensation. The substituents of **2** are listed in Table 1. The *trans*-stereochemistry of the propenone of **2** was confirmed by the coupling constant (15-16 Hz) of the alkene protons.

The measurement of these analogs' IL-5 inhibitory activity was performed by comparing the cell proliferation of Y16 cells with/without the test compounds according to known procedures (Min *et al.*, 1999). The results of the biological screening of chalcone analogs against IL-5 are listed in Table I as % inhibition at 50 μM and as IC_{50} values.

Considering the activity of budesonide (70.3% inhibition at 50 μM, IC_{50} = 26.8 μM), which is used for the treatment of chronic asthma, the activity of 6-alkoxychalcones **2** appears to be very potent as shown in Table I. The



Scheme 1. Preparation of chalcones **2**

activities of **2c-j** are much more potent than **2b**, which lacks a 6-alkoxy group on A ring. This supports the hypothesis that the hydrophobic group at the 6 position of ring A enhances the inhibitory activity of **2** against IL-5. In the case of the cyclohexyl analogs, **2c** (IC_{50} = 12.6 μM) and **2d** (IC_{50} = 12.2 μM), which have smaller substituted groups, display an activity two times greater than **2e** (IC_{50} = 29.8 μM) and **2d** (IC_{50} = 27.2 μM). Among the acyclic alkoxy analogs, **2i** exhibits the most potent activity, which is comparable to that of **2c** and **2d**. Increasing the chain length of the substituted alkoxy group enhances the activity of **2**. Compound **2i** has a larger alkoxy group than the cyclohexyl analogs **2c** or **2d**. The activity trend of the acyclic analogs obviously conflicts with that of the cyclohexyl analogs. This implies that the size of alkoxy group does not correlate with the activity. However, compounds (**2c**, **2d**, **2i**) with cLog P between 4.22 and 4.67 show the most potent activity. Compounds outside of this range of cLog P have reduced activity. This indicates that the alkoxy group contributes to the cell permeability of **2** for the enhancement of activity, rather than functioning in ligand motif binding to the receptor. Thus, these alkoxy groups appear to adjust the hydrophobicity of **2** and, in this way, affect the inhibitory activity of these chalcones. The optimum alkoxy group on ring A of **2** is one that provides the cLog P of **2** in the range of 4.22 to 4.67.

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