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Preliminary communication

Structural requirement of isoflavonones for the inhibitory activity of interleukin-5

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Abstract

Sophoricoside isolated from *Sophora japonica* is a glycoside of isoflavonone as an inhibitor of interleukin (IL)-5. To identify structural requirements of this isoflavonone for its inhibitory activity against IL-5, isoflavonones, isoflavanones, and their glycosides were prepared and their inhibitory activity was tested against IL-5. Among them, 5-benzyloxy-3-(4-hydroxyphenyl)chromen-4-one (**4b**, 87.9% inhibition at 50 μM, $IC_{50} = 15.3$ μM) shows the most potent activity, comparable with that of sophoricoside. The important structural requirements of these isoflavonone analogs exhibiting the inhibitory activity against IL-5 were recognized as (1) planarity of chromen-4-one ring, (2) existence of phenolic hydroxyl at 4-position of B ring, and (3) introduction of benzyloxy at 5-position, which may act as a bulky group for occupying hydrophobic pocket in putative binding site. However the glucopyranosyl moiety of sophoricoside is not an essential motif for the activity.

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Keywords: Sophoricoside; Isoflavonones; Inhibitory activity of interleukin-5

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1. Introduction

Eosinophilic inflammation is the main histological correlate of airway hyper-responsiveness and tissue injury in the pathogenesis of bronchial asthma [1,2]. There is strong evidence for a central role of T helper type 2 (Th2) cytokines that contributes importantly to diseases such as asthma, and therapeutic strategies that target the Th2 cytokines are of potential benefit in allergic disease [3,4]. Interleukin (IL)-5 appears to be one of the main proinflammatory mediators among a growing number of cytokines and chemokines that induce eosinophilic inflammation [5,6]. IL-5 displays its cellular response by binding to a specific receptor (IL-5R), composed of two distinct polypeptides of α and β chains [7]. The IL-5Rα by itself binds to IL-5 with low affinity [8]. 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 [9]. IL-5Rβ is shared by IL-3 and GM-CSF receptors as the common signal transducer, and thereby these cytokines display several overlapping biological effects [10,11]. Rather than coordinating T-cell effector development or antibody isotype secretion by B-cells, biological effects of IL-5 are confined primarily to growth, differentiation, survival, chemotaxis and activation of eosinophils [12–14]. The contribution of IL-5 to allergic disease has been greatly facilitated 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 [15]. 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 [16].

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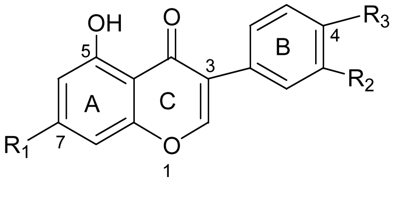
Thus, IL-5 is critically involved in eosinophilia-associated allergic inflammation.

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 possibly acting nearly without side effects. However small organic compounds to inhibit IL-5 activity have been rarely found. The isothiazolones were identified as the IL-5 antagonists through modification of Cys66 in IL-5Rα [17]. Sophoricoside and its analogs (Fig. 1) were isolated from *Sophora japonica*, a plant of Leguminosae family, as inhibitors of IL-5 bioactivity [18], and showed differential inhibition on IL-3 and GM-CSF bioactivities [19]. These isoflavonoids are unrelated to the structural unit of IL-5 and are the first natural products to show the inhibitory activity against IL-5 bioactivity. Therefore it is necessary to determine the structure activity relationship of sophoricoside analogs for the design of potent derivatives. Thus a number of isoflavonone compounds have been prepared and their inhibitory effects on IL-5 bioactivity have been evaluated.

2. Chemistry

The synthetic pathways of sophoricoside analogs are outlined in Fig. 2. Sophoricoside (**13**) is a glycoside of isoflavonone. Thus its analogs were prepared using conventional isoflavonone synthesis [20,21], which is the most convenient method considering the availability of starting material. To obtain the selectively substituted sophoricoside analogs, benzyl protected 2-hydroxyacetophenones **1** were used as starting material in initial step. 2-Hydroxyphenylpropenones **3** were readily obtained by aldol condensation between 2-hydroxyacetophenones **1** and 4-methoxymethoxybenzaldehyde (**2**) in the presence of sodium hydroxide in 90% aqueous ethanol. Sequential treatments of compounds **3** with thallium nitrate in methanol at room temperature for 24 h and then heating at 50 °C after addition of hydrochloric acid in one pot afforded the corresponding isoflavonones **4** in good yield. This conversion has been known as a key step for this classical preparation of isoflavonone [20,21]. To remove the benzyl-protecting group on A ring of **4**, two different hydrogenolysis conditions were applied in the presence of 10% palladium–carbon in methanol. Hydrogen pressure less than 20 psi only removed benzyl group to form isoflavonones **5**. However increasing hydrogen pressure to 30 psi resulted in both removal of the benzyl and reduction of 2,3-double bond to give isoflavanone **7**. Controlling hydrogen pressure in this reaction makes this synthetic scheme a versatile method for the preparation of isoflavonone and isoflavanone derivatives. Treatment of **5b** with 1 equiv. of benzyl bromide in the presence of potassium carbonate selectively gave **6b**, in which hydroxyl at 4-position of B ring is benzylated. This selectivity might be originated from the steric hinderence, thus the alkylation might occur at the less hindered hydroxyl function. Such selectivity had been demonstrated in the glycosidation of genistein to acetylated sophoricoside [22]. Glycosidation of **4** to glucopyranosylisoflavonones **8** was successfully performed by the 2-day reaction at room temperature with 2-bromoglucopyranosyl acetate and potassium carbonate in the presence of benzyl triethyl ammonium chloride as a phase transfer catalyst [23,24]. Under catalytic hydrogenolysis condition (10% Pd–C, 30 psi hydrogen), removal of the benzyl protecting group and reduction of 2,3-double bond of 4′-glycosylisoflavonone **8b** was accomplished to give isoflavanone **9b**. Meanwhile catalytic hydrogenolysis under reduced pressure of hydrogen gas (20 psi) of **8b** gave compound **10b**. Treatment of the acetates **8a** and **8b** with sodium methoxide in methanol gave the corresponding glycosides **11a** and **11b**. Benzyl group of **11b** was selectively removed by the low-pressure hydrogenolysis condition used for the preparation of **5** to produce **12b**. Sophoricoside isolated from *Sophora japonica* [17] was reduced to isoflavanone **14** by catalytic hydrogenation at 30-psi hydrogen in the presence of 10% palladium–carbon as shown in Fig. 3.



Sophoricoside	R1 = OH, R2 = H, R3 = OGlu
Genistin	R1 = OGlu, R2 = H, R3 = OH
Genistein	R1 = OH, R2 = H, R3 = OH
Orobol	R1 = OH, R2 = OH, R3 = OH

Fig. 1. Naturally occurring isoflavonoids.

3. Pharmacology

The measurement of inhibitory activity of IL-5 of these analogs was performed with the comparison of cell proliferation of Y16 cell with/without the samples [18]. An Y16 cell line originated from murine early B cell is proliferated in the presence of IL-5, which was used as the parameter of IL-5 bioactivity. Non-radioactive procedures that measure cell metabolism as an index of proliferation have now become popular. 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2*H*-

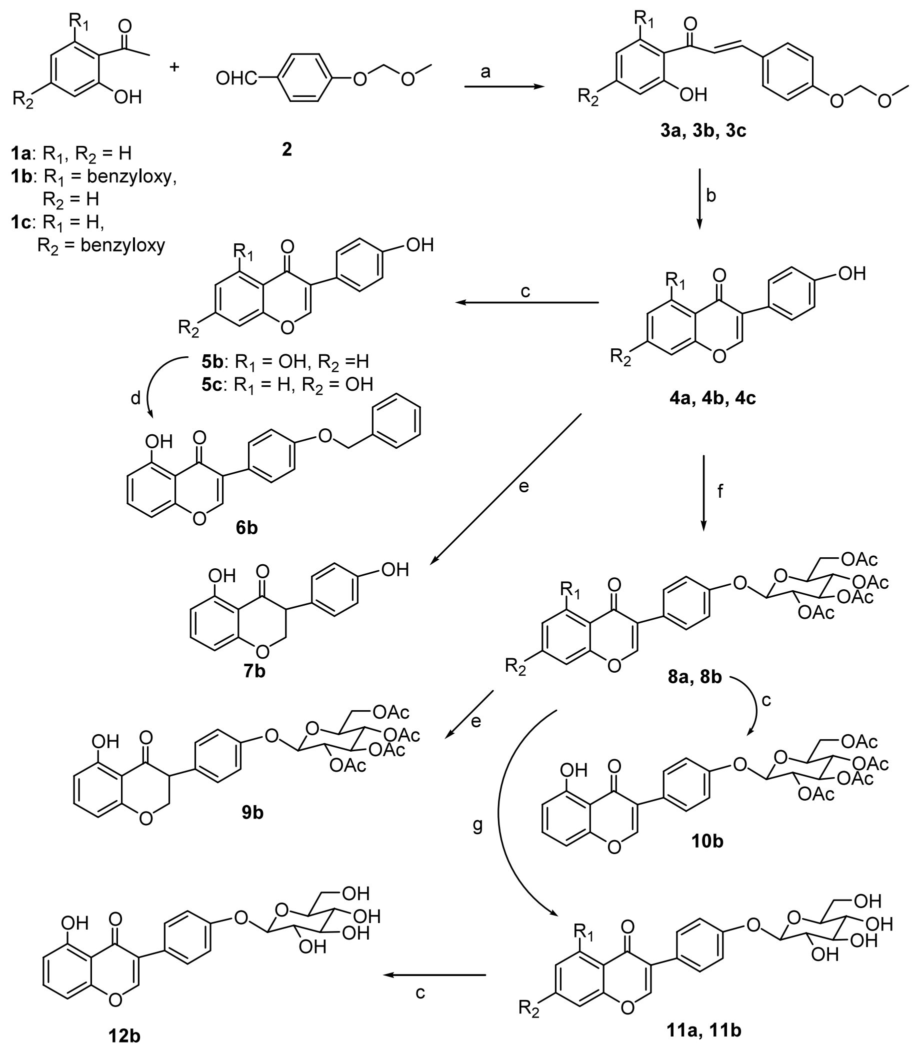


Fig. 2. Preparation of sophoricoside analogs. Substituent for compounds **3**, **5**, **8**, and **11** are the same as those of compound **1**. (a) 9% NaOH in 90% aqueous ethanol; (b) $Tl(NO_3)_2 \cdot 3H_2O$ for 1 day and then HCl in methanol; (c) 20 psi H_2, 10% Pd–C in methanol; (d) NaOH, benzyl bromide; (e) 30 psi H_2, 10% Pd–C in methanol; (f) K_2CO_3, 2-bromoglucopyranosyl acetate; (g) $NaOCH_3$ in methanol.

tetrazolium sodium salt (WST-1) was used to form soluble formazan product on exposure to the dehydrogenase activity in metabolising cells. Proliferation of Y16 cells in the presence of IL-5 was specifically blocked by treatment of polyclonal antibody against IL-5 or monoclonal antibody against IL-5Rα. Sample and IL-5 were simultaneously added to the Y16 cells, and the bioassay system can identify the IL-5 antagonist and its signal inhibitor.

4. Results and discussion

The results of biological screening of sophoricoside analogs against IL-5 are listed in Table 1 as % inhibition at 50 μM and IC_{50} values. Sophoricoside, genistin, genistein, and orobol isolated from fruit of *Sophora japonica* L. (Leguminosae) as the active principles against cytokine IL-5 [18,19]. Among them sophoricoside shows the most potent activity, which is much

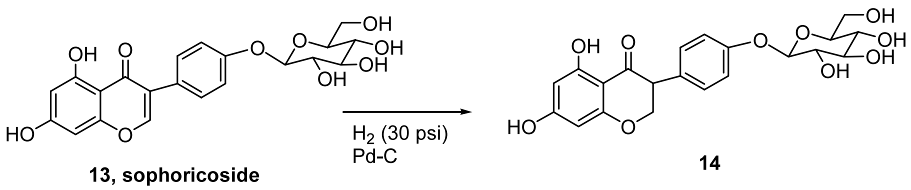


Fig. 3. Reduction of sophoricoside.

Table 1
Inhibitory activity of sophoricoside analogs against IL-5

Entry no.	Compound no.	Molecular formula	% Inhibition at 50 (μM)	IC_{50} (μM)
1	**4a**	$C_{15}H_{10}O_3$	16.2±0.3	> 50
2	**4b**	$C_{22}H_{16}O_4$	87.8±6.4	15.3
3	**4c**	$C_{22}H_{16}O_4$	57.7±1.0	36.3
4	**5b**	$C_{15}H_{10}O_4$	57.3±3.4	45.1
5	**5c**	$C_{15}H_{10}O_4$	69.6±2.8	30.6
6	**6b**	$C_{22}H_{16}O_4$	41.0±4.3	> 50
7	**7b**	$C_{15}H_{12}O_4$	17.2±3.2	> 50
8	**8a**	$C_{29}H_{28}O_{12}$	8.0±9.3	> 50
9	**8b**	$C_{36}H_{34}O_{13}$	39.4±3.9	> 50
10	**9b**	$C_{29}H_{30}O_{13}$	33.6±3.6	> 50
11	**10b**	$C_{29}H_{28}O_{13}$	55.0±3.1	35.3
12	**11a**	$C_{21}H_{20}O_8$	−5.0±2.7	> 50
13	**11b**	$C_{28}H_{26}O_9$	91.3±2.4	23.4
14	**12b**	$C_{21}H_{20}O_9$	95.3±0.4	26.1
15	**13**	$C_{21}H_{20}O_{10}$	93.0±0.8	10.3
16	**14**	$C_{21}H_{22}O_{10}$	17.6±2.9	> 50
17	Budesonide	–	70.3±2.1	26.8

stronger than oxyphenylbutazone [18]. Although removal of glucopyranosyl moiety of sophoricoside decreases the activity 34 times based on IC_{50} value comparison [18], the structure activity relationships of these isoflavonones are essentially unknown. To find out the primary pharmacophoric motif of these analogs, compounds **4a**–**c**, **5b** [25], **5c** [25], **6b**, **7b**, **8a**, **8b**, **9b**, **10b**, **11a**, **11b**, **12b**, **13** (sophoricoside), **14** were tested.

Introduction of benzyloxy moiety at 5- or 7-positions on A ring of isoflavonones increases the activity. Isoflavonones **4b** (87.9% inhibition at 50 μM, IC_{50} = 15.3 μM) and **4c** (57.7% inhibition at 50 μM, IC_{50} = 36.3 μM) containing benzyloxy at 5- or 7-position show relatively strong activity, which are comparable with sophoricoside activity (90.0% inhibition at 50 μM, IC_{50} = 10.3 μM). However **4a** (16.2% inhibition at 50 μM, IC_{50} > 50 μM) without benzyloxy moiety on A ring exhibits very weak activity. This trend continues in the activity of compounds **8** and **11** series. Compounds **8a** and **11a** do not have benzyloxy group on A ring and show very weak activity. Whereas compounds **8b** and **11b** possessing benzyloxy on A ring show much more potent activity. Compound **4b** with benzyloxy moiety at 5-position appears more active than **4c** at the 7-position. However compound **6b** (17.2% inhibition at 50 μM, IC_{50} > 50 μM) with benzyloxy group at 4-position of B ring shows very weak activity. These results should suggest that benzyloxy moiety on A ring of isoflavonone capable to bind with hydrophobic pocket in putative binding site increases the activity.

Compounds (**5b**, **5c**, **10b**, **12b**) possessing hydroxyl groups on both A and B rings shows compatible level of activity compared to the corresponding compounds (**4b**, **4c**, **8b**, **11b**) with benzyloxy group on A ring and hydroxyl group on B ring. Compound **4a** without hydroxyl on A ring shows very weak activity. Alkylation of hydroxyl group at 4-position of B ring of isoflavonone as in **6b** reduces the activity dramatically. Therefore hydroxyl function at this position is considered to be essential for the activity. Although these hydroxyl compounds **5b**, **5c**, **10b**, and **12b** do not have hydrophobic binding moiety on A ring like benzyloxy group, increased hydrophilicity of these compounds by hydroxyl function on A ring might be a factor for the enhancement of the activity.

Since sophoricoside has stronger activity than its aglycon [18] and IL-5 is heavily glycosylated, it was assumed that the glucopyranosyl moiety has its unique role for the activity. To confirm the role of glucopyranosyl moiety at 4-hydroxyl group of B ring of isoflavonones, glucopyranosyl analogs **11b** and **12b** were prepared and their activity were compared to those of their corresponding aglycons **4b** and **5b**. Compound **11b** (91.3% inhibition at 50 μM, IC_{50} = 23.4 μM) has the same level of activity with **4b**. Compound **12b** (95.3% inhibition at 50 μM, IC_{50} = 26.1 μM) is slightly more active than compound **5b** (57.3% inhibition at 50 μM, IC_{50} = 45.1 μM). Introduction of glucopyranosyl moiety at this position dose not much affect the activity. Rapid metabolic cleavage of glycosidic linkage of isoflavonoid glycoside has been known in physiological condition [26]. This might reflect that weak glycosidic bond should be cleaved metabolically or chemically under physiological condition before binding to the receptor. Thus the activity of **11b**, **12b**, and sophoricoside (**13**) are essentially those of the corresponding aglycons **4b**, **5b**, and genistein. Therefore the glucopyranosyl moiety may not be an essential motif for the intrinsic activity of these analogs.

To identify the importance of planar chromen-4-one moiety, isoflavanones **7b**, **9b** and **14** containing chroman-4-one were prepared. These compounds show reduced activity compared to the corresponding isoflavonones **5b**, **10b**, and **13**. This result indicates that the planarity of this region should be critical for the activity.

We reported that sophoricoside (**13**) showed potent inhibitory effect on IL-5 involved in eosinophilic inflammation but did not inhibit proinflammatory cytokines such as TNF and IL-1 involved in broad spectra of inflammatory responses [19]. Among isoflavonones prepared and tested in this study, compound **4b** showed the strongest inhibitory effect ($IC_{50} = 15.3$ μM) on IL-5. Inhibitory potency of **4b** on IL-5 was significantly stronger than budesonide ($IC_{50} = 26.8$ μM) used as an inhaled corticosteroid for asthma therapy (Table 1). Like mode of action shown by sophoricoside, compound **4b** did not show inhibitory effect at concentration of 50 μM on TNF and IL-1 bioactivities. A study to address selectivity and mechanism of compound **4b** on IL-5 inhibition is in progress.

From this study, we could summarize the structural requirement of sophoricoside analogs possessing the inhibitory activity against IL-5 as (1) planarity of chromen-4-one ring, (2) existence of phenolic hydroxyl at 4-position of B ring, and (3) introduction of benzyloxy at 5-position, which may act as a bulky group for binding hydrophobic pocket in putative receptor. However the glucopyranosyl moiety of sophoricoside is not an essential motif for the activity.

5. Experimental protocols

5.1. Chemistry

Melting points (m.p.) were determined on 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 [27]. Thin-layer chromatography was performed on E Merck silica gel GF-254 precoated plates and the identification was done with UV light and colorization with spray 10% phosphomolybdic acid followed by heating. Flash column chromatography was performed with E Merck silica gel (230–400 mesh). IR spectra were recorded with Jasco IR-Report-100 IR spectrometer in cm^{-1} and corrected against peak at 1601 cm^{-1} of polystyrene. NMR spectra were measured against the peak of tetramethylsilane by JEOL JNM-EX90 NMR (89.45 MHz) and Varian Unity Inova 400 NMR (400 MHz) spectrometers. Elemental analysis was performed with EA1110 elemental analyser (CE Instrument).

5.1.1. General procedure [21,28] for the preparation of compounds *1*

2,6-Dihydroxyacetophenone (40 mmol) or 2,4-dihydroxyacetophenone was dissolved in dry acetonitrile (80 mL) and potassium carbonate (6.624 g, 48 mmol)) was added. The resulting mixture was refluxed for 1 h and then benzyl bromide (8.208 g, 48 mmol) in acetonitrile (10 mL) was dropped for 15 min. The reaction mixture was refluxed for one and half hours. After cooling to room temperature, dichloromethane (320 mL) was added and washed with 5% aqueous sodium hydroxide and water three times. The organic layer was dried with anhydrous sodium sulphate and concentrated under vacuum. The crude product was recrystallized from acetone–hexane (2:1).

5.1.1.1. 6-Benzyloxy-2-hydroxyacetophenone ***1b***. Yellow solid; $R_f = 0.53$ (hexanes:ethyl acetate = 5:1); yield 71.4%; m.p. 104.5–105.3 °C; IR (KBr) 3030, 2850, 1600, 1450 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 89.45 MHz) δ 2.62 (s, 3H), 5.13 (s, 2H), 6.47 (d, $J = 8.1$ Hz, 1H), 6.59 (d, $J = 8.1$ Hz, 1H), 7.34–7.49 (m, 6H), 13.24 (s, 1H); Anal. Calc. for $C_{15}H_{14}O_3$: C, 74.36; H; 5.82. Found: C, 74.25; H, 5.90.

5.1.1.2. 4-Benzyloxy-2-hydroxyacetophenone ***1c***. White solid; $R_f = 0.40$ (hexanes:ethyl acetate = 5:1); yield 92.9%; m.p. 116.8–117.7 °C; IR (KBr) 3030, 2850, 1620 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 89.45 MHz) δ 2.53 (s, 3H), 5.09 (s, 2H), 6.51 (m, 2H), 7.28–7.69 (m, 6H), 12.73 (s, 1H); Anal. Calc. for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.27; H, 5.92.

5.1.2. Preparation [29] of 4-methoxymethoxybenzaldehyde *2*

4-Hydroxybenzaldehyde (6.1 g, 50.0 mmol) was dissolved in dry acetonitrile (80 mL) and potassium *tert*-butoxide (6.2 g, 52.5 mmol) was added. The resulting mixture was stirred for 30 min at room temperature. After addition of 18-crown-6 (1.322 g, 5 mmol), chloromethyl methyl ether (5.636 g, 70 mmol) was added. The reaction mixture was stirred for one and a half hours and filtered. The filtrate was concentrated under vacuum and dissolved in ether. After washed with 5% aqueous sodium hydroxide and water three times, the organic layer was dried with anhydrous sodium sulphate and evaporated under vacuum. The product was very pure based on TLC and NMR spectroscopic analyses. Thus the product was used in the next step without further purification.

Brown oil; $R_f = 0.39$ (hexanes:ethyl acetate = 3:1); yield 98.1%; IR (KBr) 1700, 1600, 1500 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 89.45 MHz) δ 3.48 (s, 3H), 5.26 (s, 2H), 7.14 (d, $J = 8.8$ Hz, 2H), 7.84 (d, $J = 8.8$ Hz, 2H), 9.90 (s, 1H).

*5.1.3. General procedure for the preparation of compounds **3***

The corresponding 2-hydroxyacetophenone **1** (1 equiv.) was added to 90% aqueous ethanol solution of 2 equiv. of sodium hydroxide (8.7% w/w). Aldehyde **2** (1.02 equiv.) was then added and the resulting solution was stirred for 2 h 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 sulphate and evaporated under vacuum. The crude product was purified by flash column chromatography.

*5.1.3.1. 1-(2-Hydroxyphenyl)-3-[4-(methoxymethoxy)phenyl]propenone **3a***. Yellow solid; $R_f = 0.63$ (hexanes:ethyl acetate = 3:1); yield 78.0%; m.p. 71.3–72.8 °C; IR (KBr) 1640, 1580–1560, 1505 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 89.45 MHz) δ 3.49 (s, 3H), 5.23 (s, 2H), 6.84–7.16 (m, 4H), 7.40–7.67 (m, 4H), 7.83–8.00 (m, 2H), 12.91 (s, 1H); Anal. Calc. for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.54; H, 5.71.

*5.1.3.2. 1-(2-Benzyloxy-6-hydroxyphenyl)-3-[4-(methoxymethoxy)phenyl]propenone **3b***. Yellow solid; $R_f = 0.39$ (hexanes:ethyl acetate = 5:1); yield 65.8%; m.p. 87.6–88.5 °C; IR (KBr) 1640, 1580–1560, 1510, 1450 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 89.45 MHz) δ 3.50 (s, 3H), 5.13 (s, 2H), 5.20 (s, 2H), 6.59 (m, 2H), 6.97 (m, 4H), 7.53–7.75 (m, 8H), 13.61 (s, 1H); Anal. Calc. for $C_{24}H_{22}O_5$: C, 73.83; H, 5.68. Found: C, 73.67; H, 5.77.

*5.1.3.3. 1-(4-Benzyloxy-2-hydroxyphenyl)-3-[4-(methoxymethoxy)phenyl]propenone **3c***. Yellow solid; $R_f = 0.23$ (hexanes:ethyl acetate = 5:1); yield 61.5%; m.p. 96.4–97.3 °C; IR (KBr) 3250, 1620, 1580, 1500 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 89.45 MHz) δ 3.48 (s, 3H), 5.09 (s, 2H), 5.28 (s, 2H), 6.48–6.62 (m, 2H), 7.08 (d, $J = 11.5$ Hz, 2H), 7.25–7.68 (m, 6H), 7.82–7.95 (m, 4H), 13.50 (s, 1H); Anal. Calc. for $C_{24}H_{22}O_5$: C, 73.83; H, 5.68. Found: C, 73.72; H, 5.75.

*5.1.4. General procedure [20,21] for the preparation of compounds **4***

To the solution of **3** (3.6 mmol) in methanol (400 mL), thallium nitrate trihydrate (7.2 mmol) was added. The resulting solution was stirred at room temperature for 1 day. After concentration to about 200 mL, hydrochloric acid (2 N, 25 mL) was added and the resulting mixture was stirred at 50 °C for 5 h. After removal of insoluble material by filtration, the filtrate was concentrated at room temperature under vacuum. The crude product was dissolved in chloroform (50 mL) and washed with water three times. The organic layer was dried with anhydrous sodium sulphate and concentrated under vacuum. The crude product was purified by flash column chromatography.

*5.1.4.1. 3-(4-Hydroxyphenyl)chromen-4-one **4a***. Yellow solid; $R_f = 0.39$ (hexanes:ethyl acetate = 3:1); yield 51.3%; m.p. 178.6–179.3 °C; IR (KBr) 3300, 1640, 1620, 1540, 1460 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 89.45 MHz) δ 7.23–7.74 (m, 4H), 7.78–7.95 (m, 2H), 8.06 (s, 1H), 8.09–8.19 (m, 2H), 10.66 (s, 1H); Anal. Calc. for $C_{15}H_{10}O_3$: C, 75.62; H, 4.23. Found: C, 75.38; H, 4.35.

*5.1.4.2. 5-Benzyloxy-3-(4-hydroxyphenyl)chromen-4-one **4b***. Yellow solid; $R_f = 0.44$ (hexanes:ethyl acetate = 1:1); yield 69.3%; m.p. 161.7–162.5 °C; IR (KBr) 3400–3100, 1620, 1460 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.29 (s, 2H), 6.08 (broad s, 1H), 6.84–7.04 (m, 4H), 7.28–7.60 (m, 8H), 7.84 (s, 1H); Anal. Calc. for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.55; H, 4.81.

*5.1.4.3. 7-Benzyloxy-3-(4-hydroxyphenyl)chromen-4-one **4c***. Yellow solid; $R_f = 0.47$ (hexanes:ethyl acetate = 1:1); yield 80.1%; m.p. 228.4–229.7 °C; IR (KBr) 3400–3000, 2850, 1620, 1510, 1440 cm^{-1}; ^{1}H-NMR (acetone-d_6, 89.45 MHz) δ 5.31 (s, 2H), 6.72–7.02 (m, 2H), 7.08–7.21 (m, 2H), 7.34–8.13 (m, 8H), 8.18 (s, 1H), 8.43 (broad s, 1H); Anal. Calc. for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.57; H, 4.79.

*5.1.5. General procedure for the preparation of compounds **5***

Compound **4** (0.3 mmol) was dissolved in methanol (25 mL) and 10% Pd–C (15 mg) was added. The resulting mixture was stirred under hydrogen gas (20 psi) at room temperature for 2 h. After removal of catalyst by filtration with aid of celite pad, the solvent was removed under vacuum. The crude product was purified by flash column chromatography.

*5.1.5.1. 5-Hydroxy-3-(4-hydroxyphenyl)chromen-4-one **5b** [25]*. Yellow solid; $R_f = 0.24$ (hexanes:ethyl acetate = 3:1); yield 63.7%; m.p. 151.5–153.0 °C; IR (KBr) 3400, 2925, 1650, 1610, 1580, 1520, 1470 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.03 (broad s, 1H), 6.83 (d, $J = 4.0$ Hz, 1H), 7.10 (m, 4H), 7.42 (dd, $J = 2.0$, 6.4 Hz, 2H), 7.55 (t, $J = 8.4$ Hz, 1H), 7.96 (s, 1H); Anal. Calc. for $C_{15}H_{10}O_4$: C, 70.89; H, 3.96. Found: C, 70.69; H, 4.14.

*5.1.5.2. 7-Hydroxy-3-(4-hydroxyphenyl)chromen-4-one **5c** [25]*. White solid; $R_f = 0.36$ (hexanes:ethyl acetate = 1:1); yield 84.0%; m.p. 344.1–345.6 °C; IR (KBr) 3400–3000, 1630, 1520, 1460 cm^{-1}; ^{1}H-NMR (CD_3OD, 89.45 MHz) δ 6.79–7.00 (m, 4H), 7.37 (m, 2H), 8.06 (s, 1H), 8.13 (s, 1H); Anal. Calc. for $C_{15}H_{10}O_4$: C, 70.89; H, 3.96. Found: C, 70.71; H, 4.08.

*5.1.6. Preparation of 3-(4-benzyloxyphenyl)-5-hydroxychromen-4-one **6b***

Compound **5b** (160 mg, 0.63 mmol) was dissolved in dry acetonitrile (40 mL) and potassium carbonate (95 mg, 0.69 mmol) was added. The resulting mixture was stirred at 60 °C for 1 h and benzyl bromide (108 mg, 0.63 mmol) was added. After 2 h stirring at 60 °C, the solvent was removed under vacuum. Chloroform (50 mL) was added to the residue and washed with water three times. The organic layer was dried with anhydrous sodium sulphate and concentrated under vacuum. The crude product was purified by flash column chromatography.

Yellow solid; $R_f = 0.45$ (hexanes:ethyl acetate = 3:1); yield 68.1%; m.p. 145.2–146.0 °C; IR (KBr) 3600–3200, 1640, 1605, 1580, 1500, 1470 cm^{-1}; ^1H-NMR ($CDCl_3$, 89.45 MHz) δ 5.11 (s, 2H), 6.77–7.10 (m, 4H), 7.18–7.61 (m, 8H), 7.96 (s, 1H), 12.68 (s, OH); Anal. Calc. for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.62; H, 4.75.

*5.1.7. Preparation [30] of 5-hydroxy-3-(4-hydroxyphenyl)chroman-4–one **7b***

Compound **6b** (90 mg, 0.2614 mmol) was dissolved in methanol (50 mL) and 10% Pd–C (50 mg) was added. The resulting mixture was stirred under hydrogen (30 psi) for 2 h at room temperature. After removal of catalyst by filtration with aid of celite pad, the solvent was removed under vacuum. The crude product was purified by flash column chromatography.

White solid; $R_f = 0.23$ (hexanes:ethyl acetate = 5:1); yield 68.7%; m.p. 97.5–98.7 °C; IR (KBr) 3600–3100, 2900, 1650, 1520, 1460 cm^{-1}; ^1H-NMR($CDCl_3$, 89.45 MHz) δ 3.96 (t, $J = 6.9$ Hz, 1H), 4.63 (d, $J = 6.9$ Hz, 2H), 6.42–6.59 (m, 2H), 6.76–7.11 (m, 2H), 7.20–7.55 (m, 4H), 11.81 (s, 1H); Anal. Calc. for $C_{15}H_{12}O_4$: C, 70.31; H, 4.27. Found: C, 70.15; H, 4.32.

*5.1.8. General procedure [23,24] for the preparation of compounds **8***

Compound **5a** or **5b** (0.25 mmol) and 2-bromoglucopyranosyl acetate (3 equiv.) were dissolved in chloroform (6 mL). To the resulting solution, benzyl triethyl ammonium chloride (0.2 equiv.), potassium carbonate (6 equiv.) and water (5% w/w of potassium carbonate) were added. The resulting mixture was stirred for 2 days. After addition of chloroform (40 mL), the organic layer was washed with water three times, dried with anhydrous sodium sulphate, and concentrated under vacuum. The crude product was purified by flash column chromatography.

*5.1.8.1. 3-[4-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyloxy)phenyl]chromen-4-one **8a**.* Yellow solid; $R_f = 0.27$ (hexanes:ethyl acetate = 1:1); yield 65.0%; m.p. 173.2–174.4 °C; IR (KBr) 3100–2900, 1750 cm^{-1}; ^1H-NMR($CDCl_3$, 400 MHz) δ 2.05 (s, 3H). 2.06 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 3.87–3.92 (m, 1H), 4.25–4.28 (m, 2H), 5.15–5.22 (m, 2H), 5.31–5.34 (m, 2H), 7.42–7.54 (m, 4H), 7.72–7.76 (m, 1H), 7.82 (dd, $J = 2.4$, 8.8 Hz, 1H), 8.02 (d, $J = 2.0$ Hz, 1H), 8.09 (s, 1H), 8.31 (dd, $J = 1.4$, 7.8 Hz, 1H); Anal. Calc. for $C_{29}H_{28}O_{12}$: C, 61.27; H, 4.96. Found: C, 61.01; H, 5.13.

*5.1.8.2. 5-Benzyloxy-3-[4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy)phenyl]chromen-4-one **8b**.* White solid; $R_f = 0.31$ (hexanes:ethyl acetate = 1:1); yield 82.9%; m.p. 159.0–160.1 °C; IR (KBr) 3100–2850, 1750, 1640, 1600, 1500, 1450 cm^{-1}; ^1H-NMR ($CDCl_3$, 400 MHz) δ 2.04 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 2.09 (s, 3H), 3.87 (m, 1H), 4.18 (m, 1H), 4.30 (m, 1H), 5.10 (m, 1H), 5.17 (m, 1H), 5.28 (s, 2H), 5.31 (m, 2H), 6.86–7.07 (m, 4H), 7.26–7.61 (m, 8H), 7.83 (s, 1H); Anal. Calc. for $C_{36}H_{34}O_{13}$: C, 64.09; H, 5.08. Found: C, 63.87; H, 5.17.

*5.1.9. Preparation of 5-benzyloxy-3-[4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy)phenyl]chroman-4-one **9b***

Procedure used for the preparation of **7b** was utilized for the preparation of **9b** starting with **8b** (80 mg, 0.12 mmol).

Yellow solid; $R_f = 0.50$ (hexanes:ethyl acetate = 1:1); yield 85.9%; m.p. 67.8–68.9 °C; IR (KBr) 3300, 2950, 1750 cm^{-1}; ^1H-NMR ($CDCl_3$, 400 MHz) δ 2.04 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 3.85 (m, 1H), 4.00 (dd, $J = 5.8$, 8.6 Hz, 1H), 4.16 (d, $J = 12.4$ Hz, 1H), 4.28 (dd, $J = 4.4$, 13.2 Hz, 1H), 4.61 (t, $J = 6.8$ Hz, 2H), 5.07 (d, $J = 7.2$ Hz, 1H), 5.16 (t, $J = 9.6$ Hz, 1H), 5.29 (m, 1H), 6.47 (d, $J = 8.0$ Hz, 1H), 6.54 (d, $J = 8.8$ Hz, 1H), 7.00 (d, $J = 8.8$ Hz, 2H), 7.23 (d, $J = 8.4$ Hz, 2H), 7.39 (t, $J = 8.2$ Hz, 1H), 11.70 (d, $J = 2.0$ Hz, 1H); Anal. Calc. for $C_{29}H_{30}O_{13}$: C, 59.38; H, 5.16. Found: C, 59.18; H, 5.25.

*5.1.10. Preparation of 5-hydroxy-3-[4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy)phenyl]chromen-4-one **10b***

Procedure used for the preparation of **5b** was utilized for the preparation of **10b** starting with **8b** (80 mg, 0.12 mmol).

Yellow solid; $R_f = 0.36$ (hexanes:ethyl acetate = 1:1); yield 78.8%; m.p. 98.2–99.5 °C; IR (KBr) 3500–2950, 1750, 1650–1680, 1510 cm^{-1}; ^1H-NMR ($CDCl_3$, 400 MHz) δ 2.05 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 2.09 (s, 3H), 3.75 (m, 1H), 4.19 (dd, $J = 2.4$, 12.4 Hz, 1H), 4.31 (m, 1H), 5.12 (m, 1H), 5.18 (m, 1H), 5.30 (m, 2H), 6.83 (d, $J = 8.0$ Hz, 1H), 6.93 (d, $J = 8.0$ Hz, 1H), 7.09 (d, $J = 4.4$ Hz, 2H), 7.48 (d, $J = 4.4$ Hz, 2H), 7.56 (t, $J = 8.4$ Hz, 1H), 7.97 (s, 1H), 12.60 (s, 1H); Anal. Calc. for $C_{29}H_{28}O_{13}$: C, 59.59; H, 4.82. Found: C, 59.45; H, 4.94.

5.1.11. General procedure for the preparation of compounds *11*

Acetate **8a** or **8b** (0.54 mmol) was dissolved in methanol (10 mL) and sodium methoxide (5 equiv.) was then added. The resulting solution was stirred for 1 h at room temperature. After removal of solvent under vacuum, the crude product was purified by flash column chromatography.

5.1.11.1. 3-[4-(β-D-Glucopyranosyloxy)phenyl]chromen-4-one ***11a***. white solid; $R_f = 0.54$ (chloroform:methanol = 3:1); yield 57.9%; m.p. 103.5–104.5 °C; IR (KBr) 3600–3000, 1640, 1540 cm^{-1}; ^{1}H-NMR (dimethylsulphoxide-d_6, 400 MHz) δ 3.30 (m, 1H), 3.70 (m, 1H), 4.58 (m, 3H), 5.14 (m, 3H), 5.32 (d, $J = 4.4$ Hz, 1H), 5.38 (d, $J = 4.4$ Hz, 1H), 7.00 (m, 1H), 7.40 (d, $J = 8.8$ Hz, 1H), 7.54 (m, 2H), 7.80 (m, 3H), 8.00 (d, $J = 8.0$ Hz, 1H), 8.17 (m, 1H), 8.71 (s, 1H); Anal. Calc. for $C_{21}H_{20}O_8$: C, 63.00; H, 5.03. Found: C, 62.85; H, 5.14.

5.1.11.2. 5-Benzyoxy-3-[4-(β-D-glucopyranosyloxy)phenyl]chromen-4-one ***11b***. Yellow solid; $R_f = 0.45$ (chloroform:methanol = 5:1); yield 82.0%; m.p. 93.5–94.6 °C; IR (KBr) 3500–3000, 1600 cm^{-1}; ^{1}H-NMR (DMSO, 400 MHz) δ 3.26–3.50 (m, 3H), 3.71(d, $J = 11.2$ Hz, 2H), 4.12 (broad s, 1H), 4.91 (d, $J = 7.6$ Hz, 1H), 4.99 (d, $J = 7.2$ Hz, 1H), 5.26 (s, 2H), 5.22–5.33 (m, 3H), 7.07–7.19 (m, 3H), 7.32–7.52 (m, 5H), 7.62 (d, $J = 7.6$ Hz, 2H), 7.70 (t, $J = 8.4$ Hz, 2H), 8.31 (s, 1H); Anal. Calc. for $C_{28}H_{26}O_9$: C, 66.40; H, 5.17. Found: C, 66.23; H, 5.32.

5.1.12. Preparation of 5-hydroxy-3-[4-(β-D-glucopyranosyloxy)phenyl]chromen-4-one *12b*

Procedure used for the preparation of **5b** was utilized for the preparation of **12b** starting with **11b** (70 mg, 0.14 mmol).

White solid; $R_f = 0.59$ (chloroform:methanol = 3:1); yield 79.9%; m.p. 84.3–85.4 °C; IR (KBr) 3600–3000, 1630, 1500, 1460 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.19–3.47 (m, 3H), 3.71 (m, 2H), 4.60 (broad s, 1H), 4.84 (m, 2H), 4.93 (d, $J = 6.8$ Hz, 1H), 5.06 (broad s, 1H), 5.36 (broad s, 1H), 6.85 (d, $J = 8.4$ Hz, 1H), 7.12 (m, 3H), 7.54 (d, $J = 8.8$ Hz, 2H), 7.69 (t, $J = 8.4$ Hz, 1H), 8.57 (s, 1H), 12.65 (broad s, 1H); Anal. Calc. for $C_{21}H_{20}O_9$: C, 60.58; H, 4.84. Found: C, 60.45; H, 4.96.

5.1.13. Preparation of 5,7-dihydroxy-3-[4-(β-D-glucopyranosyloxy)phenyl]chroman-4-one *14*

Procedure used for the preparation of **7b** was utilized for the preparation of **14** starting with sophoricoside (**12**, 100 mg, 0.23 mmol) isolated from *Sophora japonica* [18]. The reaction was run for 15 h.

White solid; $R_f = 0.41$ (chloroform:methanol = 3:1); yield 94.5%; m.p. 82.3–83.4 °C; IR (KBr) 3600–3000, 1640, 1500 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.15 (m, 2H), 3.24 (m, 3H), 3.32 (m, 2H), 3.46 (m, 2H), 3.67 (m, 1H), 3.74 (s, 1H), 4.06 (t, $J = 6.6$ Hz, 1H), 4.54 (d, $J = 5.2$ Hz, 2H), 4.84 (d, $J = 7.6$ Hz, 1H), 5.82 (d, $J = 4.8$ Hz, 2H), 7.00 (d, $J = 8.8$ Hz, 2H), 7.20 (d, $J = 8.8$ Hz, 2H), 12.19 (s, 1H); Anal. Calc. for $C_{21}H_{22}O_{10}$: C, 58.06; H, 5.10. Found: C, 57.93; H, 5.24.

5.2. Inhibitory activity against interleukin-5

The Y16 cell line was grown in RPMI (10.4 mg mL^{-1} RPMI-1640, 24 mM $NaHCO_3$, 100 units mL^{-1} benzylpenicillin potassium, 100 μg mL^{-1} streptomycin sulphate, pH 7.1) containing 8% fetal bovine serum (FBS) and 5 units mL^{-1} IL-5 at 37 °C with 5% CO_2. The grown Y16 cells were harvested by centrifugation at $250 \times g$ for 10 min at 4 °C, washed twice with Hanks' solution (9.8 mg mL^{-1} Hanks' balanced salts, 24 mM $NaHCO_3$, pH 7.1), and resuspended in a small volume of RPMI containing 8% FBS. Number of the cells were counted after trypan blue exclusion and then diluted to 1×10^5 cells mL^{-1} with RPMI containing 8% FBS. Viability of the cells was more than 95% in all preparations. One hundred μL of 1×10^4 cells were dispensed to each well of a 96-well microplate (Nunc, Denmark), and 50 μL of 4 units mL^{-1} IL-5 and 50 μL of the sample were added. Control group was treated with RPMI containing 8% FBS instead of sample, and blank group with RPMI containing 8% FBS instead of IL-5. After incubation at 37 °C with 5% CO_2 for 48 h, 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 methylsulphate per mL of phosphate buffered saline), and incubated 37 °C with 5% CO_2 for 4 h. Absorbance at wavelength 450 nm (A_{450}) was measured by using a microplate reader (Molecular Device, USA) Inhibitory effect on the IL-5 bioassay by sample at 50 μM concentration was expressed as % inhibition, [1-(sample A_{450} − blank A_{450})/(control A_{450} − blank A_{450})] × 100. Data were collected as mean ± S.E.M. of three independent tests. Samples showing more than 50% inhibition at 50-μM concentration were serially diluted, and then subjected to the IL-5 bioassay to determine IC_{50} values.

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