Structure-activity Relationships of 4-Senecioyloxymethyl-6,7-dimethoxycoumarin Analogues as Anti-Allergic Agents

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Mast cells are key effector cells in the early phase allergic inflammation and in diverse immunological and pathological processes. In order to understand the effect on reduction of the anti-dinitrophenyl (DNP) IgE antibody-induced β-hexosaminidase release in RBL-2H3 rat mast cells, a novel series of 4-senecioyloxymethyl-6,7-dimethoxycoumarins (SMDC) was prepared by reacting 4-chloromethyl-6,7-dimethoxycoumarin with various carboxylic acids. Compounds **8**-**11** with cyclic moiety such as phenyl, thiophenyl, pyridinyl, and furanyl group were found to inhibit-hexosaminidase release more potently (5.98-9.62 μM) than compounds **3**-**7** and **12** with acyclic moiety (19.32-76.78 μM). Furthermore, compounds **8** and **9** inhibited IgE-induced ear swelling and significantly reduced systemic passive cutaneous anaphylaxis reaction in mice.

Key Words : Atopic dermatitis, Mast cells, β-Hexosaminidase, Coumarin analogues

Introduction

Atopic dermatitis (AD) is a chronic or chronically relapsing inflammatory skin condition that primarily affects children. AD is a common, chronic, relapsing, inflammatory skin disease characterized by typically distributed eczematous skin lesions with lichenification, pruritic excoriations, severely dry skin, and a susceptibility to cutaneous infections.[1] Mast cells participate in the pathogenesis of several inflammatory skin disorders such as AD. They are mononuclear, granule-containing secretory cells that reside mostly in the skin and increase in number in chronic AD lesions.[2] Mast cell activation brings about the process of degranulation, which results in the fusion of the cytoplasmic granule membranes with the plasma membrane.[3] Both histamine and β-hexosaminidase stored in mast cells are effectively released by allergen. Mast cells express on their surface membrane receptors with high affinity and specificity for IgE.

Crinum latifolium L. (Amaryllidaceae) is a herbal drug in Vietnamese and Chinese traditional medicine for its antitumor and immunomodulatory activity.[4-9] However, to the best the author's knowledge, there is no report on the active compounds showing antitumor and immunomodulatory activity isolated from the this plant. It was only reported that

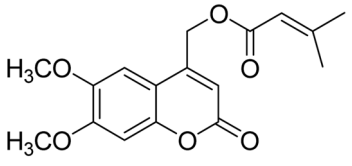


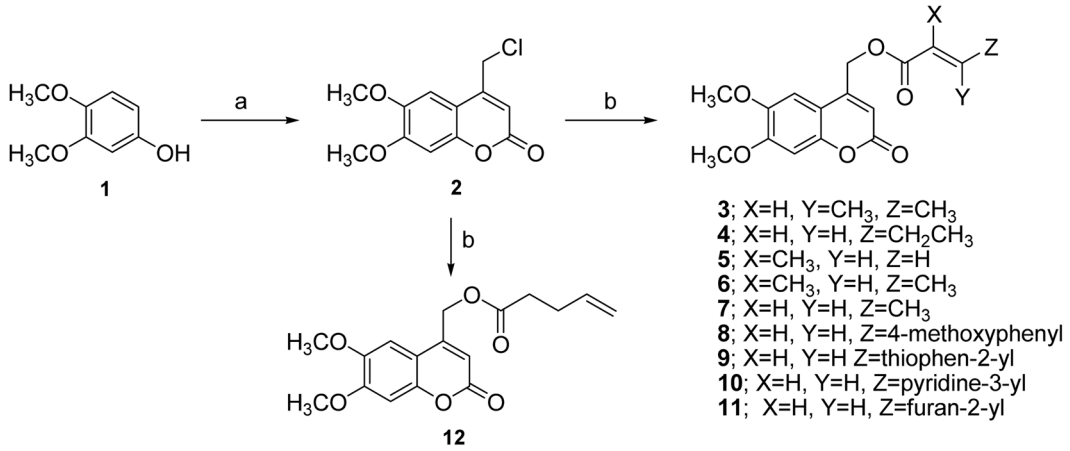
Figure 1. Structure of 4-senecioyloxymethyl-6,7-dimethoxycoumarin (**3**, SMDC).

4-senecioyloxymethyl-6,7-dimethoxycoumarin (**3**, SMDC, Figure 1), isolated from *Crinium latifolium*, exhibited antiangiogenic activity without any cytotoxicity.[10,11]

Recently, we found that SMDC reduced the anti-dinitrophenyl (DNP) IgE antibody-induced β-hexosaminidase release in RBL-2H3 cells.[12] In addition, SMDC inhibited an nuclear factor κB (NF-κB) activation and extracellular signal-regulated kinase (ERK) 1/2 activity, implying that SMDC might be useful for the clinical application to treat allergic diseases such as atopic dermatitis.[12] This results prompted a further investigation to find out the structure-activity relationships of SMDC analogues on effect of the inhibition of β-hexosaminidase release in rat mast cells sensitized with anti-DNP IgE. Herein we describe the initial structure-activity relationship (SAR) of SMDC analogues.

For the SAR study, a series of SMDC analogues was prepared containing simple alkyl groups and cyclic groups such as phenyl, thiophenyl, pyridinyl, and furanyl in the side-chain moiety of the C-4 of coumarin ring. Scheme 1 outlines the preparation of SMDC analogues. For the synthesis of target compounds **3**-**11**, we utilized the key intermediate, 4-chloromethyl-6,7-dimethoxycoumarin (**2**), which was obtained by reacting 3,4-dimethoxyphenol (**1**) with ethyl-4-chloroacetoacetate in the presence of excess sulfuric acid using Pechmann condensation condition.[13] Treatment of compound **2** with various carboxylic acids containing acyclic moiety in dry acetonitrile allowed **3**-**7** and **12**, respectively. Reaction of compound **2** with various carboxylic acids containing cyclic moiety such as phenyl, thiophenyl, pyridinyl, and furanyl afforded **8**-**11**, respectively.

To evaluate the immune-suppressive property of the compounds **3**-**12**, all compounds were initially examined for



Scheme 1. Reagents and conditions: (a) Ethyl-4-chloroacetoacetate, conc-H_2SO_4 (excess), 1 h, 0 °C, then 30 min, rt; (b) Carboxylic acid (excess), triethylamine (TEA), dry CH_3CN, rt, 3-12 h.

Table 1. Inhibitory effect of SDMC analogues on β-hexosaminidase activity

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
3	19.32 ± 0.5 [a]	**8**	5.98 ± 0.2
4	44.87 ± 0.1	**9**	7.10 ± 0.5
5	50.45 ± 0.2	**10**	9.62 ± 0.4
6	76.78 ± 1.1	**11**	6.56 ± 0.4
7	43.54 ± 0.5	**12**	31.76 ± 0.2

[a]Mean ± S.D.

the inhibitory activity of β-hexosaminidase release in rat mast cells sensitized with anti-DNP IgE for 20 h. Table 1 shows the inhibitory effect of SMDC analogues on β-hexosaminidase release. Compound **3**, SMDC, exhibited moderate inhibitory activity (IC_{50} = 19.32 μM) while other SMDC analogues **4**-**7** and **12** with acyclic moieties showed weak inhibitory activity (IC_{50} = 43.54-76.78 μM) on β-hexosaminidase release.

Interestingly, compounds **8**-**11** with cyclic moieties such as phenyl (**8**), thiophenyl (**9**), pyridinyl (**10**), and furanyl (**11**) group exhibited potent inhibitory activity (IC_{50} = 5.98-9.62 μM). Based on these observations, we postulate the existence of the cyclic moieties at the C-4 of coumarin ring is important for the inhibition of enzyme release. The reason for this result, at this stage, is not clear. Among them, compound **8** with 4'-methoxycinnamoyl group showed the strongest inhibition activity (IC_{50} = 5.98 μM).

The effect of compound **8** and **9** was then investigated on *in vivo* passive cutaneous anaphylaxis. As demonstrated in Figure 2, it was found that the oral administration (100 mg/kg) of compound **8** and compound **9** exhibited 48% and 65% inhibition of IgE-induced ear swelling and significantly reduced systemic passive cutaneous anaphylaxis reaction in mice, respectively.

In summary, we have synthesized a series of SMDC analogues that have various acyclic and cyclic groups in the side-chain moiety of the C-4 of coumarin ring. SMDC analogues with alkyl group showed weak inhibitory activity

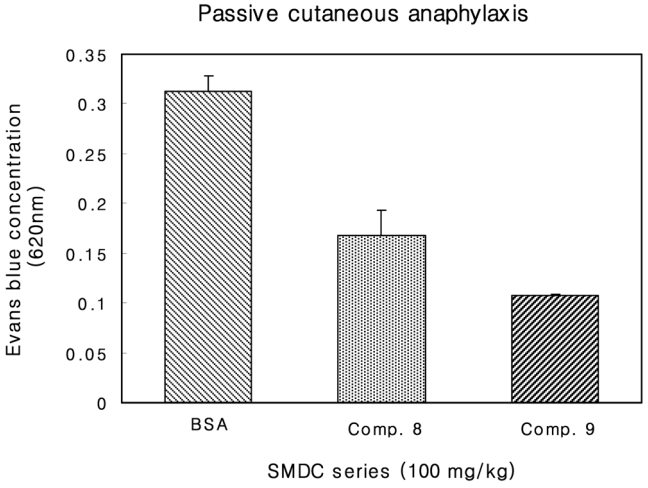


Figure 2. Effect of compound **6** and **7** on systemic passive cutaneous anaphylaxis reaction in mice.[16]

in β-hexosaminidase release while SMDC analogues with cyclic group exhibited potent inhibitory activity. Furthermore, compound **8** and **9** inhibited IgE-induced ear swelling and significantly reduced systemic passive cutaneous anaphylaxis reaction in mice. Taken together, these findings suggested that SMDC analogues with aryl group have an anti-allergic activity and potential for the treatment of allergic diseases such as atopic dermatitis.

Experimental Section

All chemicals were purchased from Aldrich and used as received. NMR spectra were performed on a JEOL JNM-AL 400 spectrometer at 400 MHz for ^{1}H NMR spectra and 100 MHz for ^{13}C NMR spectra. Melting points were recorded on a Yamako MD-S3. Low resolution mass spectra were acquired using a PE SCIX API 2000 MS/MS.

4-(Chloromethyl)-6,7-dimethoxy-2H-chromen-2-one (2). To a mixture of 3,4-dimethoxyphenol (**1**, 1 g, 6.48 mmol) and ethyl-4-chloroacetoacetate (13.2 mL, 97.3 mmol) at 0 °C

was added dropwise an excess amount of concentrated H_2SO_4 (5 mL) for 30 minutes. After stirred at room temperature for 1 h, the reaction mixture was poured into ice water. The resulting precipitate was filtrated, washed with water and dried to give compound **2** (1.528 g, 92.8%) as a yellow solid: mp 205-206 °C; R_f= 0.23 (hexanes:EtOAc = 2:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.018 (s, 1H), 6.873 (s, 1H), 6.417 (s, 1H), 4.627 (s, 2H), 3.960 (s, 3H), 3.950 (s, 3H); *m/z* 255 $(M+H)^+$.

General procedure for condensation of coumarin analogue with carboxylic acids. To a solution of 4-chloromethyl-6,7-dimethoxycoumarin (**2**, 100 mg, 0.393 mmol) and carboxylic acids (1.18-1.97 mmol) in dry acetonitrile was added triethylamine (1.18-1.97 mmol). After refluxing for 3-12 h, the reaction mixture was cooled to room temperature, then poured into saturated $NaHCO_3$ solution, and then extracted with methylene chloride (50 mL). The organic layer was dried over sodium sulfate anhydrous, filtrated, and concentrated under the reduced pressure. The residue was purified over a silica gel column eluting with gradient EtOAc with hexanes to afford the target compounds.

3-Methyl-but-2-enoic acid 6,7-dimethoxy-2-oxo-2H-chromen-4-ylmethyl ester (3). Yield 71.2%; yellow solid; mp 169-170 °C; R_f= 0.31 (hexanes:EtOAc = 2:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.866 (s, 2H), 6.383 (s, 1H), 5.811 (s, 1H), 5.289 (s, 2H), 3.951 (s, 3H), 3.917 (s, 3H), 1.965 (s, 3H); *m/z* 319 $(M+H)^+$.

Pent-2-enoic acid 6,7-dimethoxy-2-oxo-2H-chromen-4-ylmethyl ester (4). Yield 81.7%; yellow solid; mp 109-110 °C; R_f= 0.45 (hexanes:EtOAc = 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.179 (m, 1H), 6.871 (s, 1H), 6.856 (s, 1H), 6.410 (s, 1H), 5.932 (d, J = 15.6 Hz, 1H), 5.332 (s, 2H), 3.955 (s, 3H), 3.921 (s, 3H), 2.288 (m, 2H), 1.115 (t, J = 7.2 Hz, 3H); *m/z* 319 $(M+H)^+$.

2-Methyl-acrylic acid 6,7-dimethoxy-2-oxo-2H-chromen-4-ylmethyl ester (5). Yield 65.4%; yellow solid; mp 129-131 °C; R_f= 0.41 (hexanes:EtOAc = 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.872 (d, J = 4.4 Hz, 2H), 6.393 (s, 1H), 6.261 (s, 1H), 5.714 (s, 1H), 5.351 (s, 1H), 3.959 (s, 3H), 3.924 (s, 3H), 2.028 (s, 3H); *m/z* 305 $(M+H)^+$.

2-Methyl-but-2-enoic acid 6,7-dimethoxy-2-oxo-2H-chromen-4-ylmethyl ester (6). Yield 71.3%; yellow solid; mp 115-116 °C; R_f= 0.41 (hexanes:EtOAc = 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.035 (m, 1H), 6.900 (s, 1H), 6.892 (s, 1H), 6.430 (s, 1H), 5.369 (s, 2H), 3.990 (s, 3H), 3.954 (s, 3H), 1.947 (s, 3H), 1.880 (m, 3H); *m/z* 319 $(M+H)^+$.

But-2-enoic acid 6,7-dimethoy-2-oxo-2H-chromen-4-ylmethyl ester (7). yield 54.6%; yellow solid; mp 136-137 °C; R_f= 0.36 (hexanes:EtOAc = 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.126 (m, 1H), 6.872 (s, 1H), 6.860 (s, 1H), 6.391 (s, 1H), 5.966 (d, J = 15.2 Hz, 1H), 5.326 (s, 2H), 3.953 (s, 3H), 3.918 (s, 3H), 1.949 (d, J = 6.0 Hz, 3H); *m/z* 305 $(M+H)^+$.

3-(4-Methoxy-phenyl)-acrylic acid 6,7-dimethoxy-2-oxo-2H-chromen-4-ylmethyl ester (8). Yield 36.9%, white solid; mp 192-193 °C; R_f= 0.18 (hexanes:EtOAc = 2:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.762 (d, J = 16 Hz, 1H), 7.515 (d, J = 8.4 Hz, 2H), 6.929 (d, J = 8.8 Hz, 2H), 6.886 (d, J = 5.2 Hz, 2H), 6.456 (s, 1H), 6.401 (d, J = 16.0 Hz, 1H), 5.392 (s, 2H), 3.930 (s, 3H), 3.883 (s, 3H), 3.855 (s, 3H); ^{13}C MNR ($CDCl_3$, 400 MHz) δ 166.35, 161.85, 161.11, 152.96, 149.71, 149.29, 146.39, 146.32, 130.04, 126.63, 114.45, 113.84, 110.41, 109.61, 103.99, 100.33, 61.15, 56.49, 56.34, 55.40; *m/z* 397 $(M+H)^+$.

3-Thiophen-2-yl-acrylic acid 6,7-dimethoxy-2-oxo-2H-chromen-4-ylmethyl ester (9). Yield 75.0%; white solid; mp 201-202 °C; R_f= 0.21 (hexanes:EtOAc = 2:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.909 (d, J = 16.0 Hz, 1H), 7.447 (d, J = 5.2 Hz, 1H), 7.329 (d, J = 3.2 Hz, 1H), 7.095 (q, J = 2.9 Hz, 1H), 6.884 (s, 1H), 6.880 (s, 1H), 6.455 (s, 1H), 6.333 (d, J = 15.6 Hz, 1H), 5.395 (s, 2H), 3.963 (s, 3H), 3.937 (s, 3H); ^{13}C MNR ($CDCl_3$, 400 MHz) δ 165.87, 161.05, 152.98, 149.71, 149.10, 146.40, 139.03, 138.93, 131.76, 129.34, 128.27, 115.05, 110.41, 109.54, 103.95, 100.33, 61.26, 56.48, 56.34; *m/z* 373 $(M+H)^+$.

3-Pyridin-3-yl-acrylic acid 6,7-dimethoxy-2-oxo-2H-chromen-4-ylmethyl ester (10). Yield 81.7%, white solid; mp 204.0 °C; R_f= 0.072 (hexanes:EtOAc = 1:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.792 (s, 1H), 8.651 (d, J = 4.0 Hz, 1H), 7.891 (d, J = 10.0 Hz, 1H), 7.808 (d, J = 16.4 Hz, 1H), 7.376 (dd, J = 4.8, 8.0 Hz, 1H), 6.884 (d, J = 4.0 Hz, 1H), 6.623 (d, J = 16.4 Hz, 1H), 5.430 (s, 2H), 3.953 (s, 3H), 3.940 (s, 3H); *m/z* 368 $(M+H)^+$.

3-Furan-2-yl-acrylic acid 6,7-dimethoxy-2-oxo-2H-chromen-4-ylmethyl ester (11). Yield 44.3%; light yellow solid; mp 176-177 °C; R_f= 0.15 (hexanes:EtOAc = 2:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.713 (s, 1H), 7.709 (d, J = 15.6 Hz, 1H), 7.465 (s, 1H), 6.876 (d, J = 5.2 Hz, 2H), 6.629 (s, 1H), 6.447 (s, 1H), 6.257 (d, J = 16.0 Hz, 1H), 5.388 (s, 2H), 3.960 (s, 3H), 3.930 (s, 3H); *m/z* 357 (M+H).

Pent-4-enoic acid 6,7-dimethoxy-2-oxo-2H-chromen-4-ylmethyl ester (12). Yield 60.2%; yellow solid; mp 77-78 °C; R_f= 0.41 (hexanes:EtOAc = 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.870 (s, 1H), 6.844 (s, 1H), 6.376 (s, 1H), 5.848 (m, 1H), 5.278 (s, 2H), 5.057 (m, 1H), 3.954 (s, 3H), 3.920 (s, 3H), 2.578 (t, J = 7.2 Hz, 2H), 2.460 (m, 2H); *m/z* 319 $(M+H)^+$.

β-Hexosaminidase release assay. RBL-2H3 rat mast cells were obtained from the American Type Culture Collection (Bethesda, MD), and grown in MEM supplemented with 10% fetal bovine serum. Cells were sensitized with 1 μg/mL of anti-DNP IgE for 20 h and cells were incubated 500 μL of the incubation buffer (119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM PIPES, 40 mM NaOH, 5.6 mM glucose, 1 mM $CaCl_2$ containing 0.1% BSA) for 10 min at 37 °C. After that, test sample solution was added to each well and incubated for 10 min, followed by an addition of antigen (DNP-BSA; 10 μg/mL) at 37 °C for 10 min to stimulate the cells to degranulate. Aliquots (250 μL) of the incubation medium were incubated with 250 μL of 1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in 0.1 M sodium citrate buffer (pH 4.5) at 37 °C for 30 min. The absorbance was measured with a microplate reader at 405 nm.

Systemic passive cutaneous anaphylaxis assay. Ten ng

of anti-DNP IgE diluted in 10 μL of PBS was injected intradermally in both ears of ICR female mice (25-30 g). One day later, DNP-BSA was injected i.v. in 200 μL of PBS with 0.5% Evans blue. Thirty minutes after challenge, both ears were cut and incubated at 80 ^{o}C in 1 mL of formamide for 2 h. The mixture was homogenized and centrifuged at 20,800 × g for 10 min. The absorbance of the supernatant was measured at 620 nm.

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