



A new flavonoid from *Stellera chamaejasme* L., stechamone, alleviated 2,4-dinitrochlorobenzene-induced atopic dermatitis-like skin lesions in a murine model

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ABSTRACT

Stellera chamaejasme L. (family Thymelaeaceae), also known as ‘Langdu’, has been traditionally used to treat of skin-related diseases, such as, psoriasis and skin ulcers. The aim of this study was to identify the biologically active component of *S. chamaejasme* and evaluate its preventive effects on IL-4 and mast cell degranulation in RBL-2H3 cells and on the development of atopic dermatitis (AD) in 2,4-dinitrochlorobenzene (DNCB)-treated SKH-1 hairless mice. A novel flavonoid, genkwaniin 5-O-xylosyl(1 → 2)glucoside (named stechamone), and three known compounds (umbelliferone, luteolin, and luteolin-7-O-glucoside) were isolated from the aerial parts of *S. chamaejasme* using chromatographic methods. Of these four compounds, stechamone most potently inhibited IL-4 production and mast cell degranulation in RBL-2H3 cells. Topical application of 0.5% stechamone improved atopic skin symptoms, including, erythema (redness), pruritus (itching), exudation (weeping), excoriation (peeling), and lichenification (skin thickening) in DNCB-treated AD mice by accelerating skin barrier recovery function and suppressing inflammatory cell infiltration. In addition, stechamone attenuated DNCB-induced increases in IL-4 (an inflammatory T_H2 cytokine) expression and in serum IgE levels in our murine model of AD. DNCB induced AD-like skin lesions, but treatment with stechamone exhibited strong anti-atopic activity by regulating skin barrier function and reducing inflammatory responses. The results obtained suggest stechamone is a potential anti-atopic agent and treatment for skin inflammatory diseases.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by intense pruritus and eczematous plaques [1]. Complex interactions between genetic, pharmacological, environmental, and psychological factors contribute to the pathogenesis of AD [2]. In particular, recent studies have focused on the role played by environmental allergen-specific IgE in the pathophysiology of AD [3,4]. Atopic dermatitis begins in early infancy and nearly 80% of children with AD develop allergic airway diseases [5,6]. Furthermore, the presence of atopy in infants or children may be a risk factor of life-threatening allergic rhinitis and allergic asthma [6,7].

In AD patients, inflammatory T-cell infiltration and T cell-derived cytokine release are closely associated with skin lesions [8,9]. The

development of skin lesions in AD patients results from the sequential activations of T_H2- and T_H1-type cells [10]. Interleukin-4 (IL-4) and IL-5 (T_H2-type cytokines) and IFN-γ (T_H1-type cytokines) are known to play key pathogenetic roles in the initiation and maintenance of skin inflammation in AD [11]. The topical immunomodulator pimecrolimus (Elidel®, SDZ ASM 981) was developed to treat AD and approved for use in children as young as 2 years old [12] (Eichenfield et al., 2002). Pimecrolimus inhibits the expressions of cytokines, including, IL-4, IL-10, IL-2, and IFN-γ [13], and the use of nonsteroidal topical agents is now viewed to have considerable potential for the treatment of inflammatory skin conditions and atopic diseases [14,15].

Stellera chamaejasme L. (Thymelaeaceae) is a perennial herb that is widely distributed in northern and southwestern China [16]. The root of *S. chamaejasme*, which are called ‘Langdu’ in traditional Chinese

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medicine (TCM), are used to treat stubborn skin ulcers, psoriasis, chronic tracheitis, and tuberculosis [17]. Furthermore, recent studies have shown *S. chamaejasme* extracts possess anti-inflammatory, analgesic, and wound healing activities [18,19]. Phytochemically, *S. chamaejasme* contains various flavonoids with antioxidant, antiviral, and anti-cancer activities [20–22]. During our continuing search for plants with anti-inflammatory constituents, we found that the 95% EtOH extract of the aerial parts of *S. chamaejasme* and their bioactive component, stechamone, strongly inhibited IL-4 expression in RBL-2H3 cells. This study was undertaken to investigate the anti-atopic properties of stechamone on a 2,4-dinitrochlorobenzene (DNCB)-sensitized murine model of AD. Skin samples were subjected to histopathological examinations after different treatments, skin barrier recoveries were assessed, and IgE and IL-4 concentrations in blood were measured.

2. Material and methods

2.1. Plant material

The aerial parts of *Stellera chamaejasme* were collected in Yunnan province (Diqing prefecture, Xiang Ge Li Ra, China) and identified by Dr. Sang Woo Lee (Korea Research Institute of Bioscience and Biotechnology). The name *Stellera chamaejasme* L. has been verified by the World Checklist of Selected Plant Families (<http://www.theplantlist.org/tpl1.1/record/tro-32000469>) and is included in the Plant List (www.theplantlist.org). A voucher specimen (PNU-0023) was deposited at the Medicinal Herb Garden, Pusan National University.

2.2. General methods for compound isolation

^1H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectral data were obtained using an Agilent Superconducting FT-NMR 400–500 MHz Spectrometer. HR-ESI mass spectra were recorded on an Agilent Technologies, 6530 Accurate-Mass Q-TOF LC/MS. The HPLC unit (Shimadzu, Japan) was equipped with a pump (Model LC-20AT), a 254 nm UV detector (Model SPD-20A), and a data station (Model CBM-20A). Silica gel (230–400 mesh, Merck, Germany) and Sephadex LH-20 gel (25–100 μm mesh, Pharmacia, Sweden) were used for column chromatography.

2.3. Isolation of compounds from *S. chamaejasme*

Dried aerial parts of *S. chamaejasme* (6.11 kg) were extracted with 95% EtOH (12 L $\times$ 6) and the extract obtained was then evaporated under reduced pressure, suspended in distilled water (569.8 g in 5 L), and sequentially partitioned using *n*-hexane (12 L), ethyl acetate (12 L), and *n*-butanol (12 L). The ethyl acetate fraction (20.8 g) was then chromatographed on a Silica gel column chromatography eluted with a CH_2Cl_2 -MeOH gradient (20:1 $\rightarrow$ 100% MeOH) to yield 13 fractions (SCE1 $\sim$ SCE13). Fraction SCE3 (868.4 mg) was loaded onto a Sephadex LH-20 column and eluted with 100% MeOH, to afford compound 1 (umbelliferone, 24.7 mg). Fraction SCE5 (382.0 mg) was suspended in MeOH and filtered to obtain compound 2 (luteolin, 49.8 mg).

The *n*-butanol soluble fraction (34.4 g) was chromatographed on a Silica gel CC and eluted using a CH_2Cl_2 -MeOH (10:1 $\rightarrow$ 100% MeOH) gradient to yield 8 fractions (SCB1–SCB8). Fraction SCB7 (4.5 g) was suspended in MeOH and filtered to obtain compound 3 (luteolin 7-O-glucoside, 215.8 mg). The resulting filtrate of SCB7 was re-fractionated to produce 6 subfractions (SCB7-1–SCB7-6) by Silica gel CC a CH_2Cl_2 -MeOH (5:1 $\rightarrow$ 100% MeOH) gradient. Compound 4 (stechamone, 118.8 mg) was obtained by recrystallization of SCB7-4 (654.1 mg) from MeOH (Fig. 1A).

2.4. Measurement of IL-4 mRNA expression in RBL-2H3 cells

RBL-2H3 cells (a rat basophilic leukemia cell line), obtained from

the American Type Culture Collection (ATCC, #CRL-2256, Bethesda, MD), were cultured in minimum essential medium (MEM) with Eagle's salt containing 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin at 37 °C in a humidified 5% CO_2 atmosphere. Cells were incubated with and without isolates from *S. chamaejasme* at a concentration of 10 μM in the presence of PMA/ionomycin for 16 h. DMSO control samples were cultured with 0.1% dimethyl sulfoxide (DMSO) culture medium without PI. After harvesting cells, IL-4 mRNA levels were determined by quantitative real-time PCR (qPCR).

2.5. β -hexosaminidase release assay

RBL-2H3 cells were treated with anti-DNP IgE (anti dinitrophenyl-immunoglobulin E) and incubated for 24 h. After washing with Siraganian buffer, cells were incubated in buffer for 10 min, treated with DMSO or *S. chamaejasme* (10 μM) isolates for 10 min, and then with DNP-BSA antigen (10 $\mu\text{g}/\text{mL}$) for 20 min to stimulate degranulation. Supernatants were transferred to a 96-well plate and incubated with substrate (1 mM 4-nitrophenyl-*N*-acetyl- β -D-glucosaminide in 0.1 M citrate buffer) at 37 °C for 3 h. Absorbances were measured using a microplate reader at 405 nm.

2.6. Animals

SKH-1 hairless female mice (6 weeks old) were obtained from Orient Bio Inc. (Seongnam, Republic of Korea). Animals were housed in an air-conditioned animal room (25 $\pm$ 5 °C; 55 $\pm$ 5% RH) under a 12 h on/12 h off lighting cycle. Mice were given access to standard laboratory diet and water *ad libitum*. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85–23, revised 1996) and were approved by the Institutional Animal Care and Use Committee of KIST (Certification No. KIST-2016-011).

2.7. DNCB-induced atopic dermatitis in hairless mice and stechamone treatment

Dorsal skins of SKH-1 hairless mice were painted daily with 100 μL 1% 2,4-dinitrochlorobenzene (DNCB) (Sigma-Aldrich, Seoul) for 7 days (from days 0 to 6; sensitization period). From day 7 dorsal skins were treated with 100 μL 0.1% DNCB for 2 weeks every 3 days. Pre-designated animals were also treated with 0.5% stechamone (100 μL) twice daily from day 7 for 2 weeks; when treated on same days, stechamone was administered first and DNCB was administered 4 h later. Treatment naive control mice (CON) were treated with distilled water. On day 22, mice were sacrificed and dorsal skins and blood samples were collected for further investigation.

2.8. Histologic examination

Dorsal skins of SKH-1 hairless mice were fixed in 10% formalin for 24 h and processed for paraffin wax embedding. Sections (2–3 mm) were cut using a microtome, mounted on slides, dried overnight at 37 °C, and stained with hematoxylin and eosin. Histopathological changes were examined under a light microscope (Olympus CX31/BX51, Olympus Optical Co., Tokyo) and photographed (TE-2000U, Nikon Instruments Inc., Melville, USA).

2.9. Transepidermal water loss and skin hydration

A Tewameter TM210 (Courage and Khazaka, Cologne, Germany) and a SKIN-O-MAT (Cosmomed, Ruhr, Germany) were used to evaluate hairless mouse skins. Transepidermal water loss (TEWL) and skin hydration were measured weekly under standard conditions (25 $\pm$ 5 °C, 55 $\pm$ 5% RH).

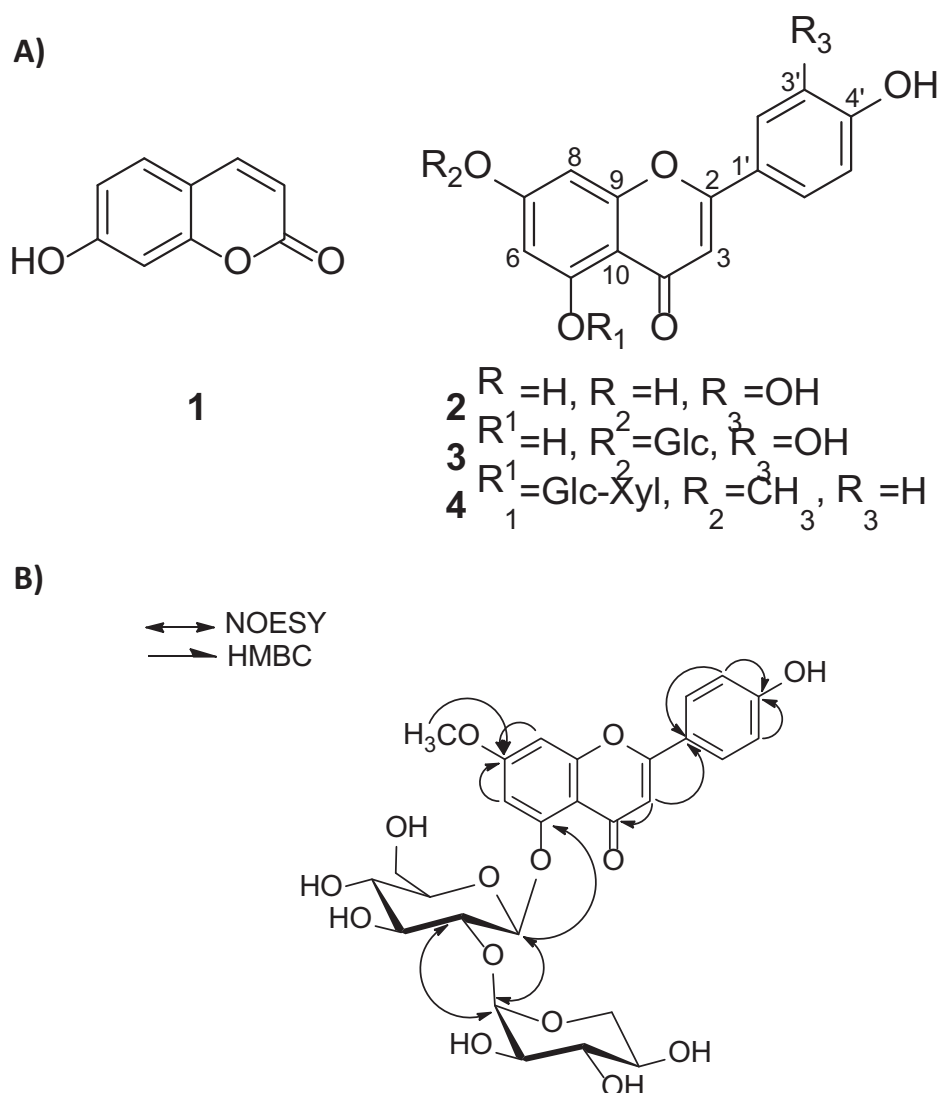


Fig. 1. Structures of the four compounds isolated from *Stellera chamaejasme*. (A) 1: umbelliferone, 2: luteolin A, 3: luteolin 7-O-glucoside, and 4: stechamone (B) Selected HMBC and NOESY correlations of stechamone.

2.10. Serum IgE and IL-4 levels

Blood samples were collected from abdominal aortas and centrifuged at 10,000 rpm for 15 min at 4 °C. Total serum IgE and IL-4 concentrations were measured using enzyme-linked immunosorbent assay kits (eBioscience, San Diego, USA) according to the manufacturer's instructions.

2.11. Statistical analysis

Results are expressed as means $\pm$ SDs. Data were analyzed using one-way analysis of variance (ANOVA) using a statistical software package. Statistical significance was accepted for p values < 0.05 .

3. Results

3.1. Structural elucidation of stechamone (compound 4)

Compound 4 was obtained as a pale yellowish powder ($[\alpha]_D^{20} -50.1$; c 0.1, MeOH) of molecular formula $C_{27}H_{30}O_{14}$, based on an m/z of 577.1626 as determined by negative-ion HRESIMS. The UV spectrum of compound 4 showed absorption at 332 nm ($\log \epsilon$ 4.29) and 261 nm

($\log \epsilon$ 4.13), which is characteristic of flavones. The IR spectrum of compound 4 displayed three significant absorption bands at 3382, 1633, and 1446 cm^{-1} indicative of hydroxyl and carbonyl groups and an aromatic ring, respectively. Analysis of its ^1H NMR data (Table 1) suggested the presence of a genkwanin moiety due to the presence of *ortho*-coupled protons assignable to a *p*-substituted phenyl moiety [δ 7.94 (2H, d, $J = 8.4$ Hz, H-2' and H-6') and 6.70 (2H, d, $J = 8.4$ Hz, H-3' and H-5')], a methoxy group at δ 3.90 (3H, s), and three aromatic singlets [δ 6.70 (1H, s, H-3), 6.89 (1H, d, $J = 2.0$ Hz, H-6), and 7.03 (1H, d, $J = 2.0$ Hz, H-8)]. The ^1H NMR spectrum of compound 4 contained signals for two anomeric protons at 4.79 (1H, d, $J = 7.6$ Hz) and 4.21 (1H, d, $J = 7.6$ Hz), suggesting the presence of a xylosylglucoside. The ^1H - ^1H COSY, HMQC, HMBC, and NOESY experiments led to the full assignments of ^1H and ^{13}C signals (Fig. 1A). The HMBC spectrum between 7-OCH₃ protons and C-7 and between H-6/H-8 and C-7 confirmed the existence of a methoxyl group at position of C-7. Furthermore, HMBC correlations between anomeric proton H-1'' (glucose) and C-5 and NOESY correlations between anomeric proton H-1''' (xylose) and H-1''/H-2'' (glucose) confirmed the positions of sugars in genkwanin. On the basis of these spectral data, compound 4 was identified as genkwanin 5-O-xylosyl(1 $\rightarrow$ 2)glucoside and named stechamone.

Table 1
 ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of stechamone (compound 4) in DMSO- d_6 .

Position	Compound 9	
	δ_{H} (J in Hz)	δ_{C}
2		161.95
3	6.70 (s)	106.46
4		177.52
5		158.76
6	6.89 (d, 2.0)	103.58
7		164.22
8	7.03 (d, 2.0)	97.27
9		159.06
10		109.79
1'		121.72
2' (6')	7.94 (d, 8.4)	128.83
3' (5')	6.70 (d, 8.4)	116.55
4'		161.52
7-OCH ₃	3.90 (s)	56.77
Glc 1	4.79 (d, 7.6)	104.39
2	3.10	77.22
3	3.30	74.03
4	3.22	70.16
5	3.22	76.57
6	3.01, 3.66	66.31
Xyl 1	4.21 (d, 7.6)	104.76
2	3.01	74.03
3	4.00	70.37
4	3.66	76.26
5	3.01, 3.66	66.31

3.2. Stechamone inhibited IL-4 expression and β -hexosaminidase release in RBL-2H3 cells

The effects of the three known compounds umbelliferone (1), luteolin (2), and luteolin 7-O-glucoside (3) and of stechamone (4) on inflammatory cytokine IL-4 expression were tested in antigen-stimulated RBL-2H3 cells. Pretreatment with luteolin, luteolin 7-O-glucoside, or stechamone for 30 min prior to stimulation with PI inhibited PI-induced increases in IL-4 mRNA expression by 31.4%, 64.3%, and 77.1%, respectively, as compared with vehicle controls (Fig. 2A). Stechamone more effectively inhibited the PI-induced up-regulation of IL-4 than the other three compounds isolated from *S. chamaejasme*. Stechamone was observed to have a similar effect on PI-induced β -hexosaminidase release (a marker of mast cell degranulation). The inhibitory effects of the four compounds on degranulation in order were: stechamone > luteolin 7-O-glucoside > luteolin. Umbelliferone did not have a significant effect on β -hexosaminidase release as compared with DNP/IgE controls.

3.3. Effects of stechamone on DNCB-induced atopic skin symptoms

The experimental procedure is summarized in Fig. 3A. On experimental day 21, severe AD-like skin lesions, such as, erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness were observed on the dorsal skins of DNCB-sensitized SKH-1 hairless mice. However, treatment with 0.5% stechamone for 14 days significantly inhibited these atopic skin symptoms (Fig. 3B). Improvements in clinical skin conditions by stechamone were confirmed by analyzing H&E-stained (Fig. 3C) or toluidine blue-stained tissue sections (Fig. 3D). H&E results showed stratum corneum thickening caused by cell hyperplasia in DNCB-treated mice was markedly attenuated by treatment with 0.5% stechamone (Fig. 4A), and toluidine blue staining showed stechamone suppressed of DNCB-induced lymphocyte infiltration and mast cell degranulation (Fig. 4B).

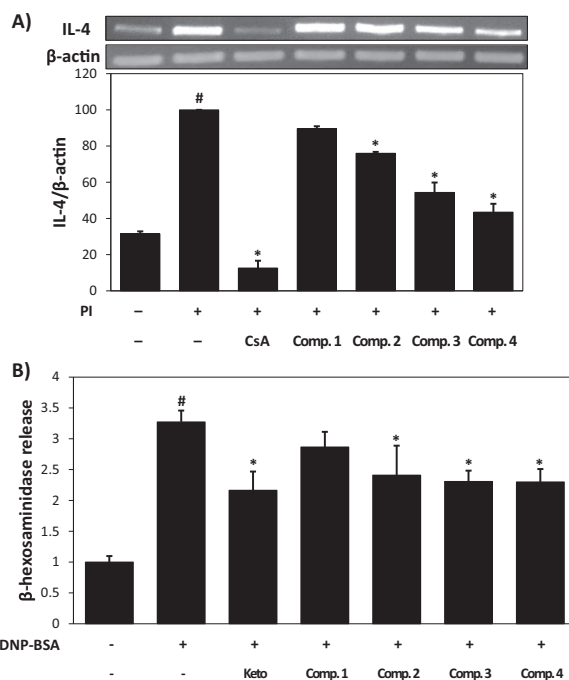


Fig. 2. Anti-inflammatory effects of the four compounds isolated from *Stellera chamaejasme* on RBL-2H3 cells. (A) Effects of compounds from *S. chamaejasme* on IL-4 mRNA expression in PI-activated RBL-2H3 cells. Results are presented as means $\pm$ SDs. The means $\pm$ SD of two independent experiments performed in triplicate are shown. (B) Effects of the compounds on β -hexosaminidase release from IgE-activated RBL-2H3 cells. Results are presented as means $\pm$ SDs. The means $\pm$ SD of two independent experiments performed in triplicate are shown. # p < 0.05 vs. vehicle controls; * p < 0.05 vs. PI. CsA: 1 μ M Cyclosporin A, Ket: 35 μ M Ketotifen.

3.4. Effects of stechamone on skin barrier function

TEWL and skin hydration were measured in DNCB-induced atopic skins as markers of skin barrier function. Treatment with DNCB significantly increased TEWL and decreased skin hydration versus treatment naive controls. After 7 days of treatment, transdermal stechamone administration decreased TEWL by 23.1% (Fig. 5A) and increased skin hydration by 26.7% (Fig. 5B) of the levels observed in DNCB-treated controls. On Day 21, TEWL values for stechamone-applied mice were remarkably lower than those of DNCB controls. In accord with these observations, stechamone also increased skin hydration levels by 39.4% as compared with the vehicle controls.

3.5. Effects of stechamone on dermal thickness and serum IgE and IL-4 levels in DNCB-induced atopic mice

Epidermal thicknesses were increased by $\sim$ 2.9-fold by DNCB treatment, and 0.5% stechamone significantly reduced DNCB-induced dermal thicknesses on day 21 (Fig. 6A). Stechamone also significantly reduced mast cell densities in dermis in DNCB-treated mice (Fig. 6B). AD is characterized by eczematous skin lesions and IgE hypersecretion, and thus, we examined the effects of stechamone on DNCB-induced IgE and IL-4 serum levels by ELISA. DNCB treatment was found to increase total serum IgE 8.8-fold and IL-4 2.1-fold in hairless mice, and 0.5% stechamone reduced these IgE and IL-4 levels to 4.0-fold and 1.1-fold, respectively.

4. Discussion

Flavonoids are the most abundant and best-studied plant polyphenols and possess many biological activities [23,24]. Furthermore, it

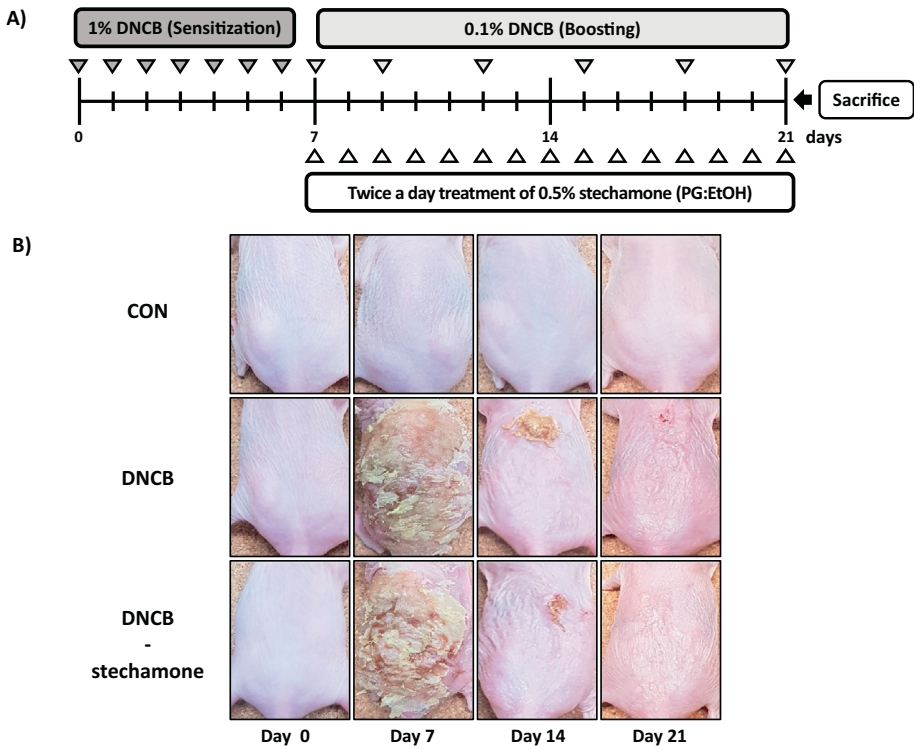


Fig. 3. Effects of stechamone on pathological changes in the skins of DNCB-sensitized atopic hairless mice. (A) Schematic representation of the experiment. (B) Clinical features of AD-like dorsal skin lesions. CON: < treatment naive controls, DNCB: DNCB-controls, DNCB-0.5% stechamone: DNCB plus 0.5% stechamone treated mice.

has been well established plants rich in flavonoids constitute a resource of natural antioxidants that help delay ageing and prevent disease [25]. Some well-known flavonoids, such as, astragalin and luteolin, have been reported to ameliorate atopic skin symptoms in murine models of AD [26,27]. These flavonoids strongly inhibit the productions of

inflammatory cytokines, such as, IL-4 and IL-13, and thus, exhibit anti-inflammatory, anti-allergic, and anti-atopic activities [28,29]. Likewise, plant-derived flavonoids have attracted much interest recently, especially in the context of AD treatment.

In a previous study, we found the 95% EtOH extract of the aerial

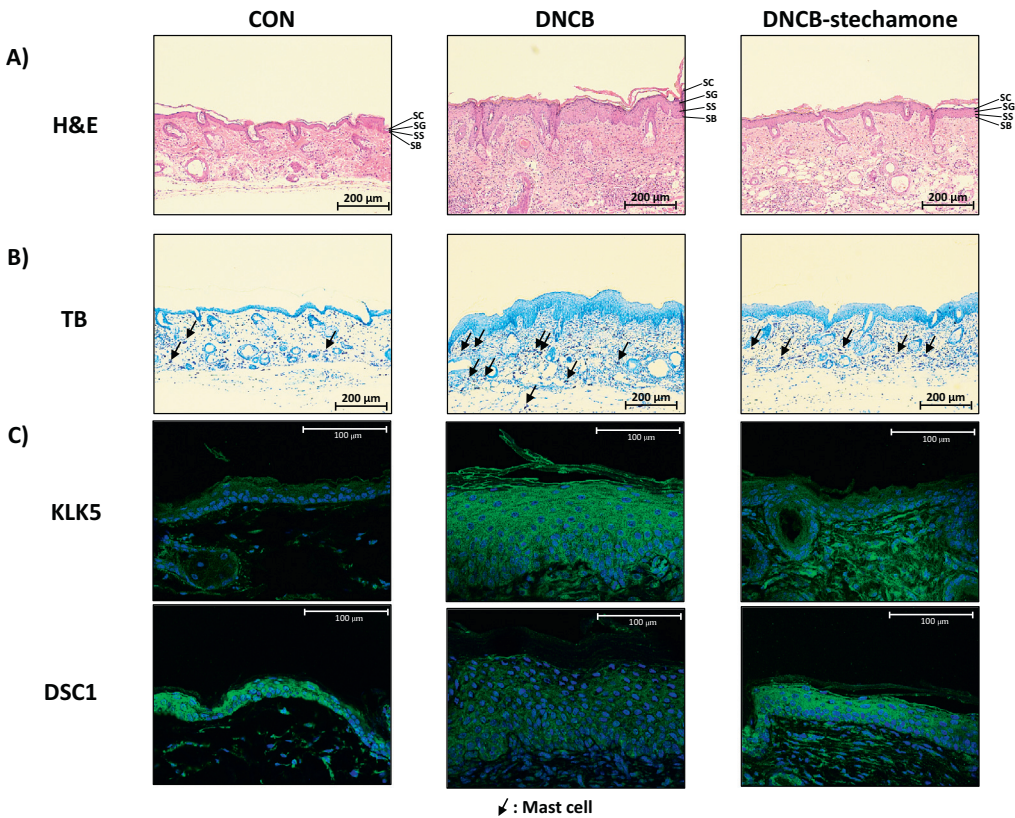


Fig. 4. Effects of stechamone on histopathological results in DNCB-sensitized atopic hairless mice. (A) Histopathological features of dorsal skin lesions in 0.5% stechamone plus DNCB treated mice as determined by H&E staining (B) Histopathological features of dorsal skin lesions in 0.5% stechamone plus DNCB co-treated mice as determined by toluidine blue staining. Tissues were excised, fixed in 10% formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) (magnification, 100×) or toluidine blue. Results are presented as means ± SDs ($n = 7$). The means ± SEMs of two independent experiments performed in triplicate are shown. [#] $p < 0.05$ vs. treatment naive controls; ^{*} $p < 0.05$ vs. DNCB treated controls.

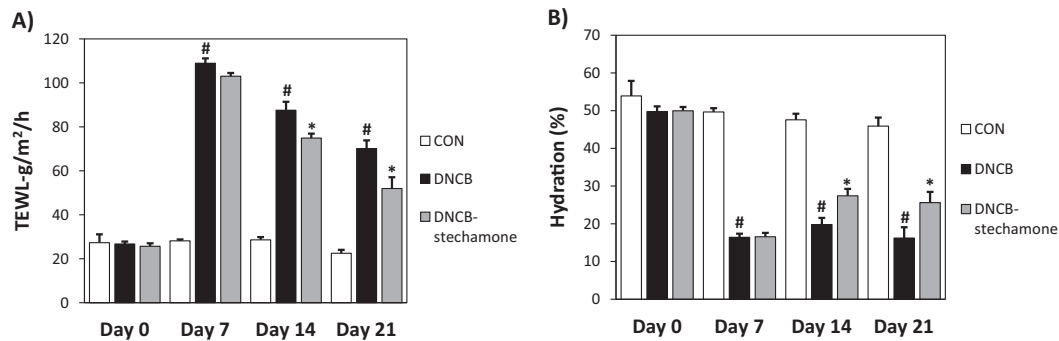


Fig. 5. Effects of stechamone on skin barrier function in DNCB-sensitized atopic hairless mice. (A) Amounts of transepidermal water loss (TEWL) (B) Estimation of skin hydration. CON: treatment naïve controls, DNCB: DNCB-controls, DNCB-stechemone: DNCB plus 0.5% stechemone treated mice. Results are presented as means $\pm$ SDs ($n = 7$). The means $\pm$ SEMs of two independent experiments performed in triplicate are shown. [#] $p < 0.05$ vs. treatment-naïve controls; ^{*} $p < 0.05$ vs. DNCB controls.

parts of *S. chamaejasme* suppressed oxazolone- and DNCB-induced atopic skin symptoms in animal models. These findings prompted us to identify components responsible and to investigate their potential uses as anti-atopic agents. In the present study, the coumarins umbelliferone and the three flavonoids luteolin, luteolin 7-*O*-glucoside, and stechemone (genkwanin 5-*O*-xylosyl(1 $\rightarrow$ 2)glucoside) were isolated from *S. chamaejasme* chromatographically. In particular, presence of genkwanin 5-*O*-xylosyl(1 $\rightarrow$ 2)glucoside named stechemone in terrestrial plants was confirmed for the first time. We then investigated the effects of these four compounds on IL-4 expression and degranulation response in RBL-2H3 mast cells. This investigation showed that stechemone most inhibited IL-4 up-regulation and mast cell degranulation in PI or IgE activated mast cells.

In our SKH-1 hairless mouse model, stechemone markedly improved

DNCB-induced atopic symptoms, such as, erythema, dryness, pruritus, excoriation, and lichenification in dorsal skin. Biophysical parameters (TEWL and skin hydration) in DNCB control suggested impairments of water retention properties and barrier functions, and treatment with 0.5% stechemone ameliorated these effects. AD is closely associated with abnormal skin barrier function and immune dysregulation [30], and topical emollients and moisturizers have been shown in clinical trials to reduce skin dryness and promote the repair of impaired skin barrier function, and thus, delay AD relapses [31,32]. Our results shown topical stechemone improves barrier functions and prevented inhibited skin disruption in our murine model of AD.

Topical corticosteroids are commonly used for the short-term management of AD [33], but although they are generally well tolerated, corticosteroids can cause severe adverse effects at sites of

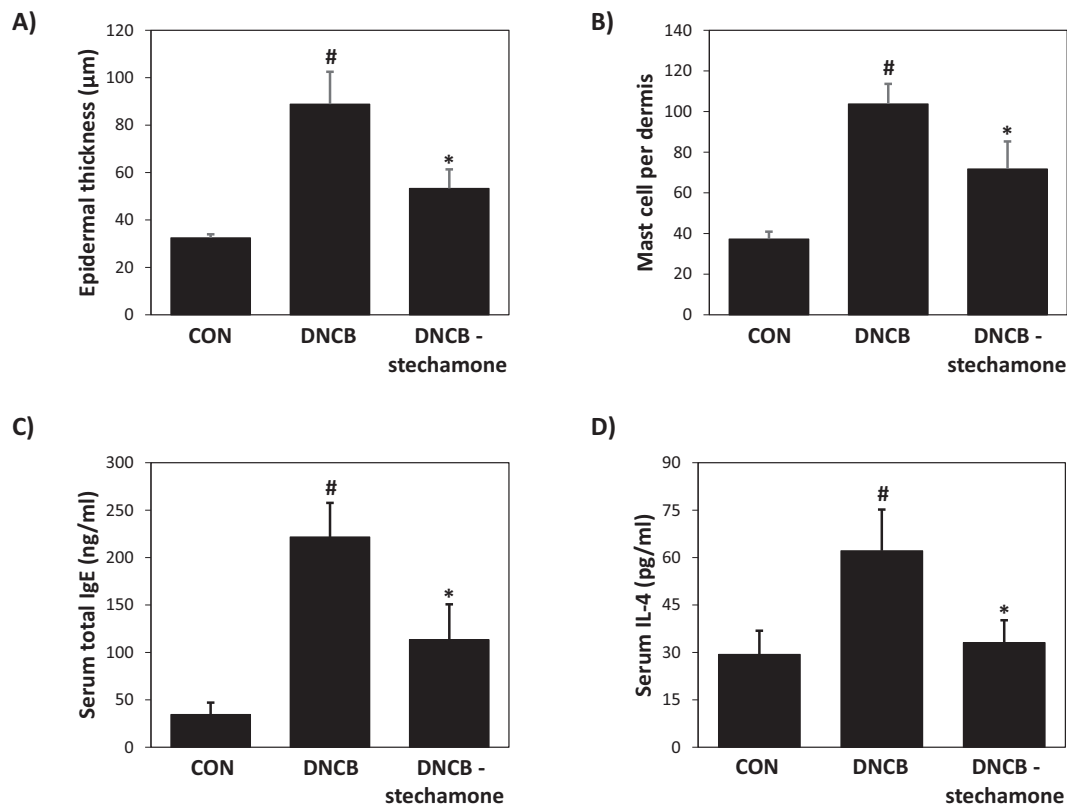


Fig. 6. Effects of stechamone on dermal thicknesses and on serum IgE and IL-4 concentrations in DNCB-induced atopic hairless mice. (A) Epidermal thickness (B) Mast cell densities in dermis (C) Total serum IgE levels (D) Total serum IL-4 levels. Results are presented as means $\pm$ standard errors ($n = 7$). The means $\pm$ SEMs of two independent experiments performed in triplicate are shown. [#] $p < 0.05$ vs. treatment naïve controls; ^{*} $p < 0.05$ vs. DNCB controls.

administration, such as, steroid-induced skin atrophy, secondary infections, and acne [34]. In contrast to corticosteroids, the topical calcineurin inhibitors, tacrolimus and pimecrolimus, were developed as maintenance therapies for AD [33,35]. Pimecrolimus selectively targets T cells and mast cells, and inhibits release of IL-2/IL-4 and mast cell degranulation. AD skin is characterized by the activation of T cells/mast cells and by the overexpression of IL-4. In the present study, stechamone was found to prevent the development of AD and to strongly inhibit DNCB-induced IL-4 expression, but did not induce any major adverse dermatological event such as skin atrophy.

5. Conclusion

In conclusion, a novel flavonoid, genkwanin 5-O-xylosyl(1 → 2) glucoside (stechamone) and three known compounds were isolated from the aerial parts of *S. chamaejasme*. *In vitro* testing using RBL-2H3 cells showed stechamone most effectively attenuated PMA/ionomycin-induced IL-4 expression and DNP/IgE-induced β -hexosaminidase release. Topical application of stechamone was observed to have anti-atopic effects in DNCB-induced AD in mice, by protecting skin barrier functions and inhibiting DNCB-induced increases in serum IgE and IL-4 levels, and DNCB-induced histopathological changes. According our results provide *in vitro* and *in vivo* evidence that support the medicinal use of stechamone for the clinical treatment of AD and related inflammatory diseases of the skin.

Authors' contributions

S.-N. Kim, and M.H. Yang designed the research. B.-G. Jo, N.-J. Park, S.-K. and J. Jegal performed experiments. B.-G. Jo, N.-J. Park, S.-N. Kim, and M.H. Yang analyzed data. B.-G. Jo, N.-J. Park, S. Choi, S. W. Lee, and H. Jin interpreted results of experiments. B.-G. Jo, N.-J. Park, S.-N. Kim, and M.H. Yang drafted manuscript. All authors read and approved the final manuscript.

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Declaration of interests

The authors declare no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2018.04.008>.

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SUPPLEMENTARY DATA

A new flavonoid from *Stellera chamaejasme* L., stechamone, alleviated 2,4-dinitrochlorobenzene-induced atopic dermatitis-like skin lesions in a murine model

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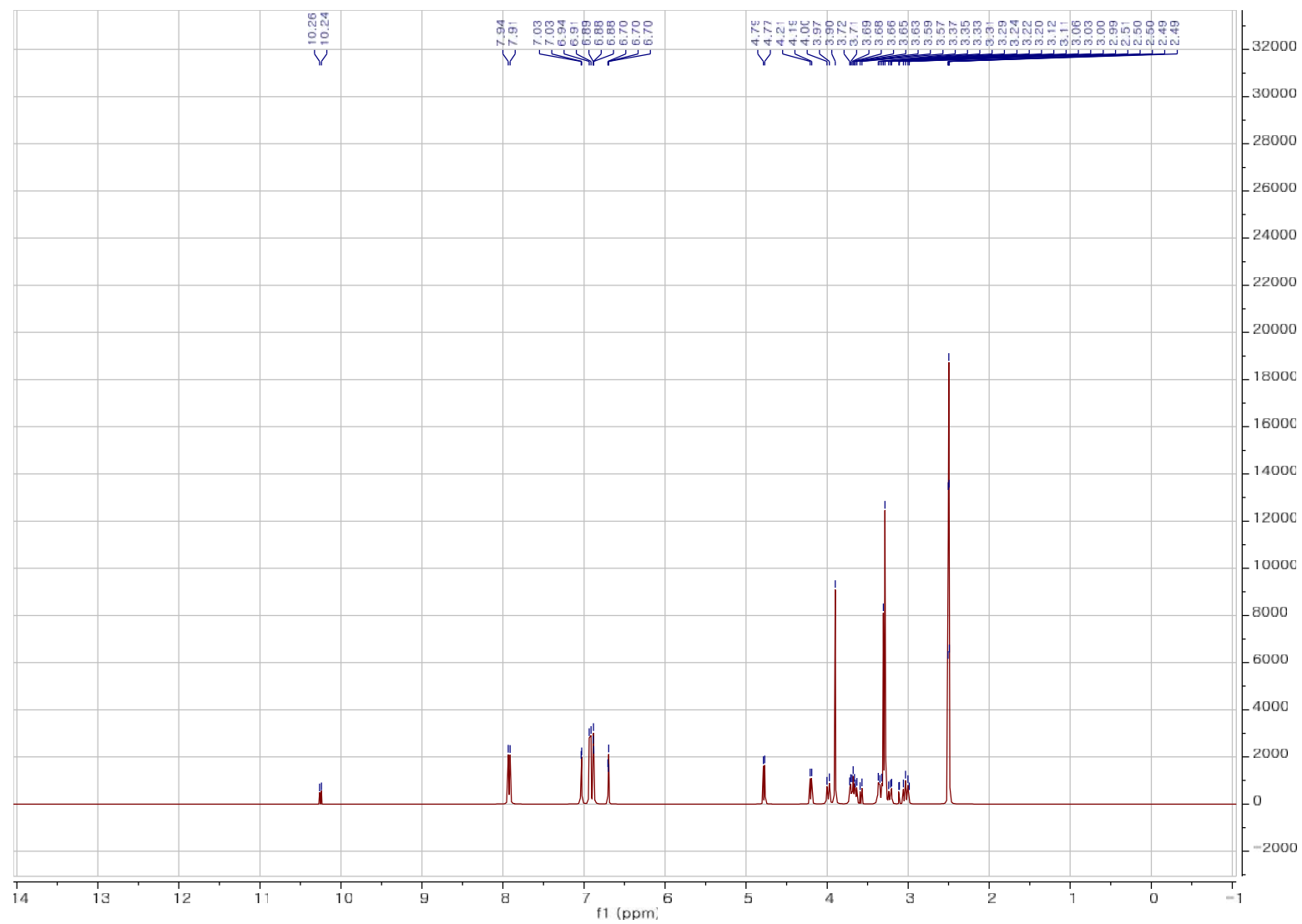
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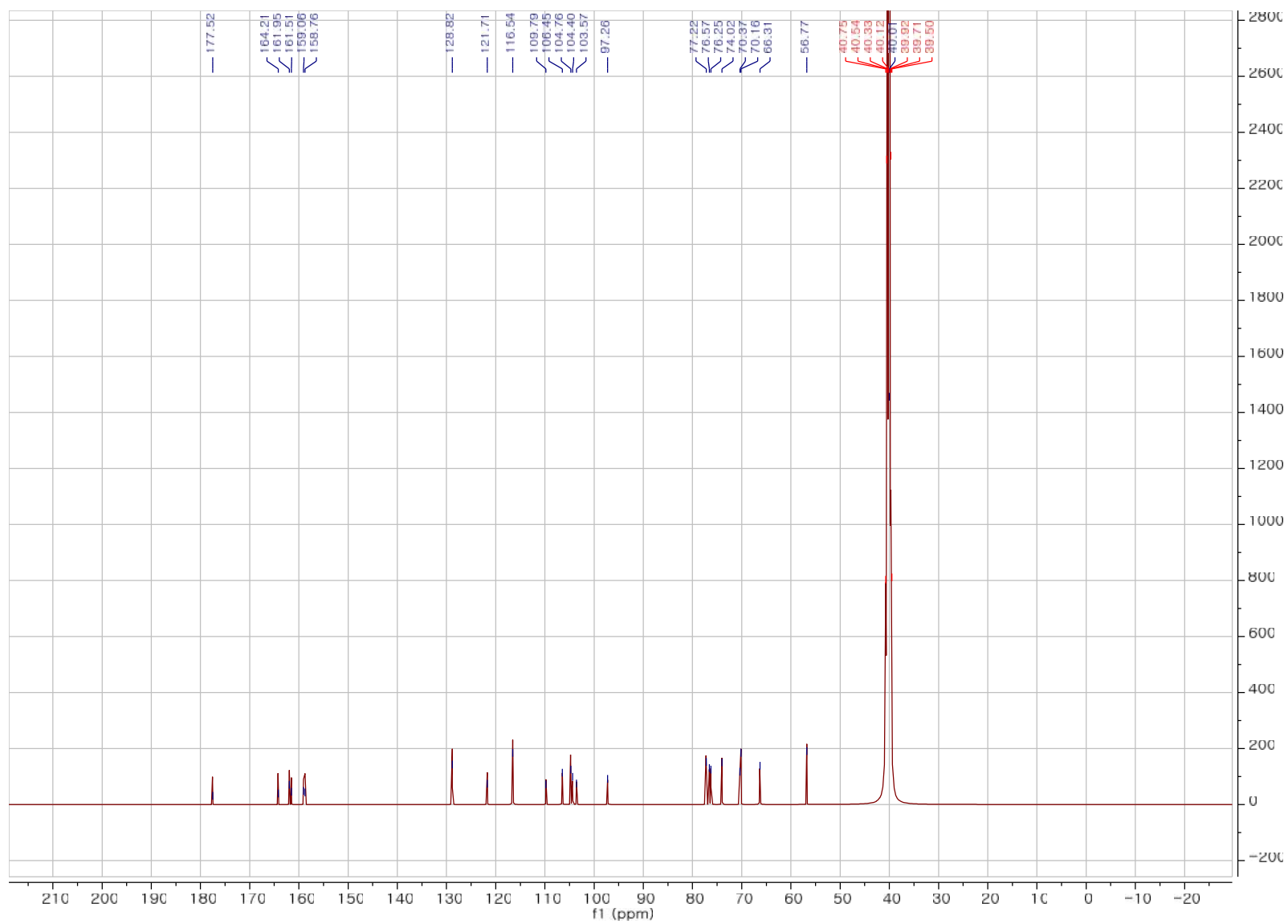
Supporting Information Contents:

- S1.** The ¹H NMR spectrum of compound **4**
- S2.** The ¹³C NMR spectrum of compound **4**
- S3.** The ¹H-¹H COSY spectrum of compound **4**
- S4.** The ¹H-¹H NOESY spectrum of compound **4**
- S5.** The HMQC spectrum of compound **4**
- S6.** The HMBC spectrum of compound **4**
- S7.** The mass spectrum of compound **4**

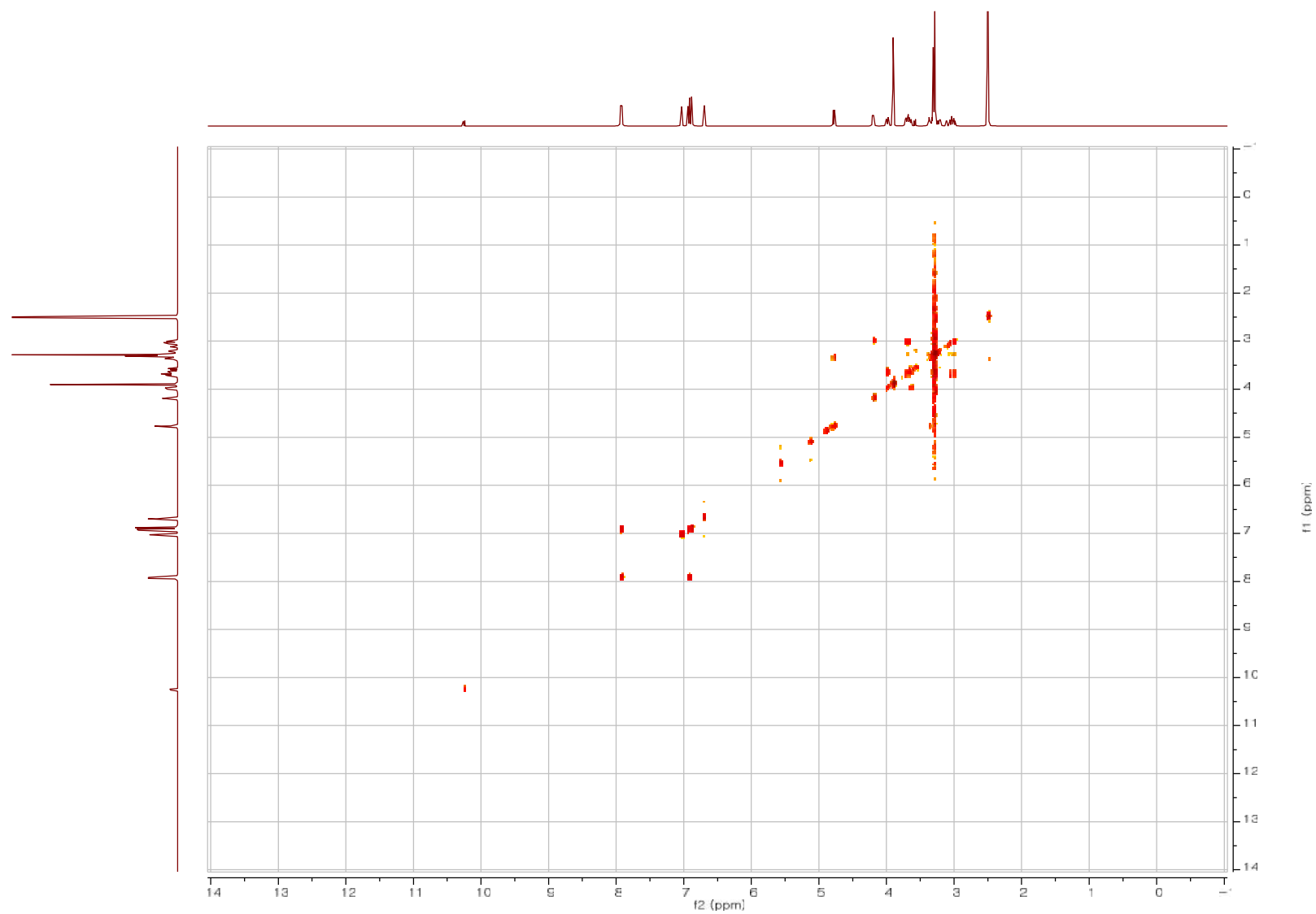
S1. The ^1H NMR spectrum of compound **4**



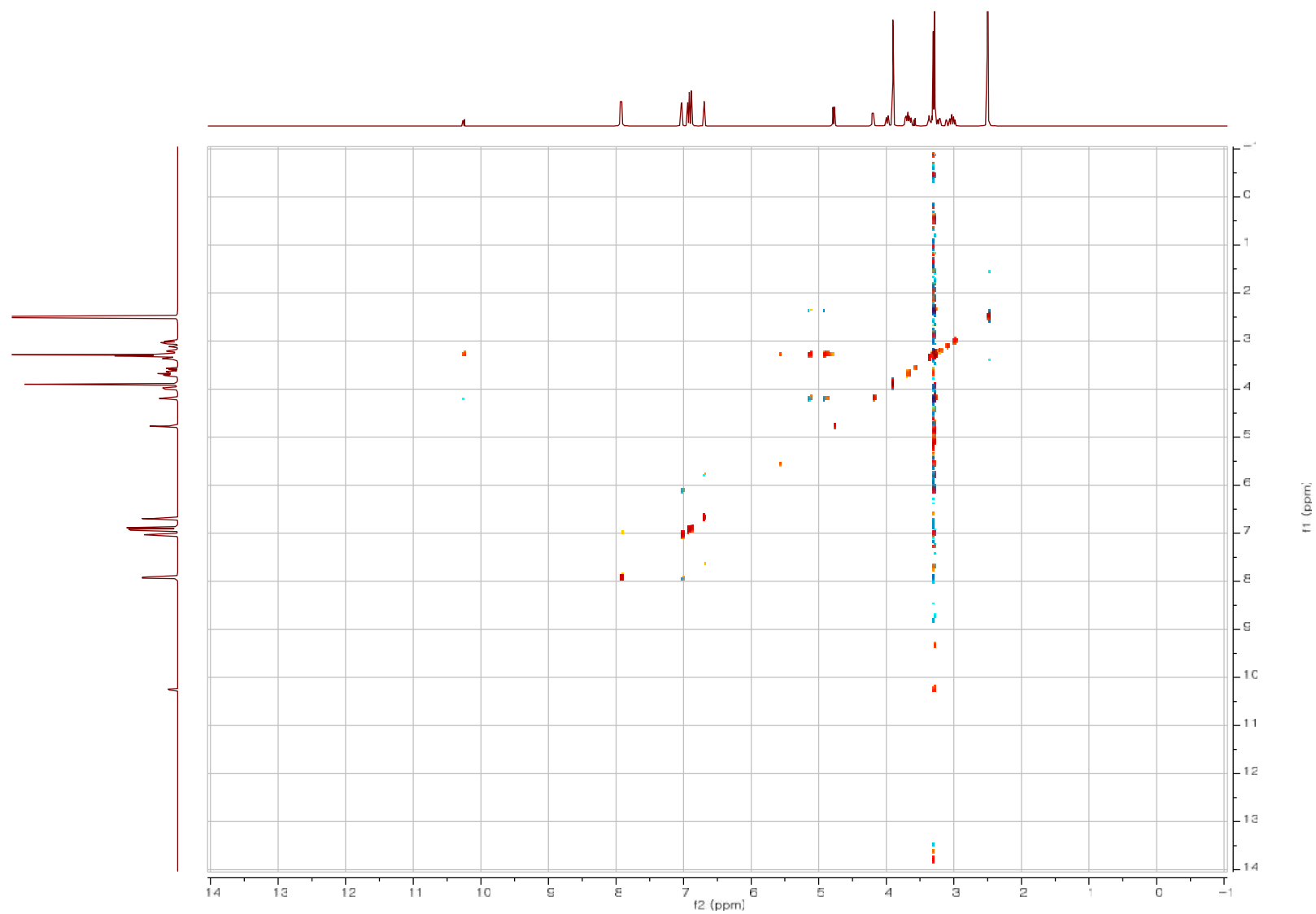
S2. The ^{13}C NMR spectrum of compound **4**



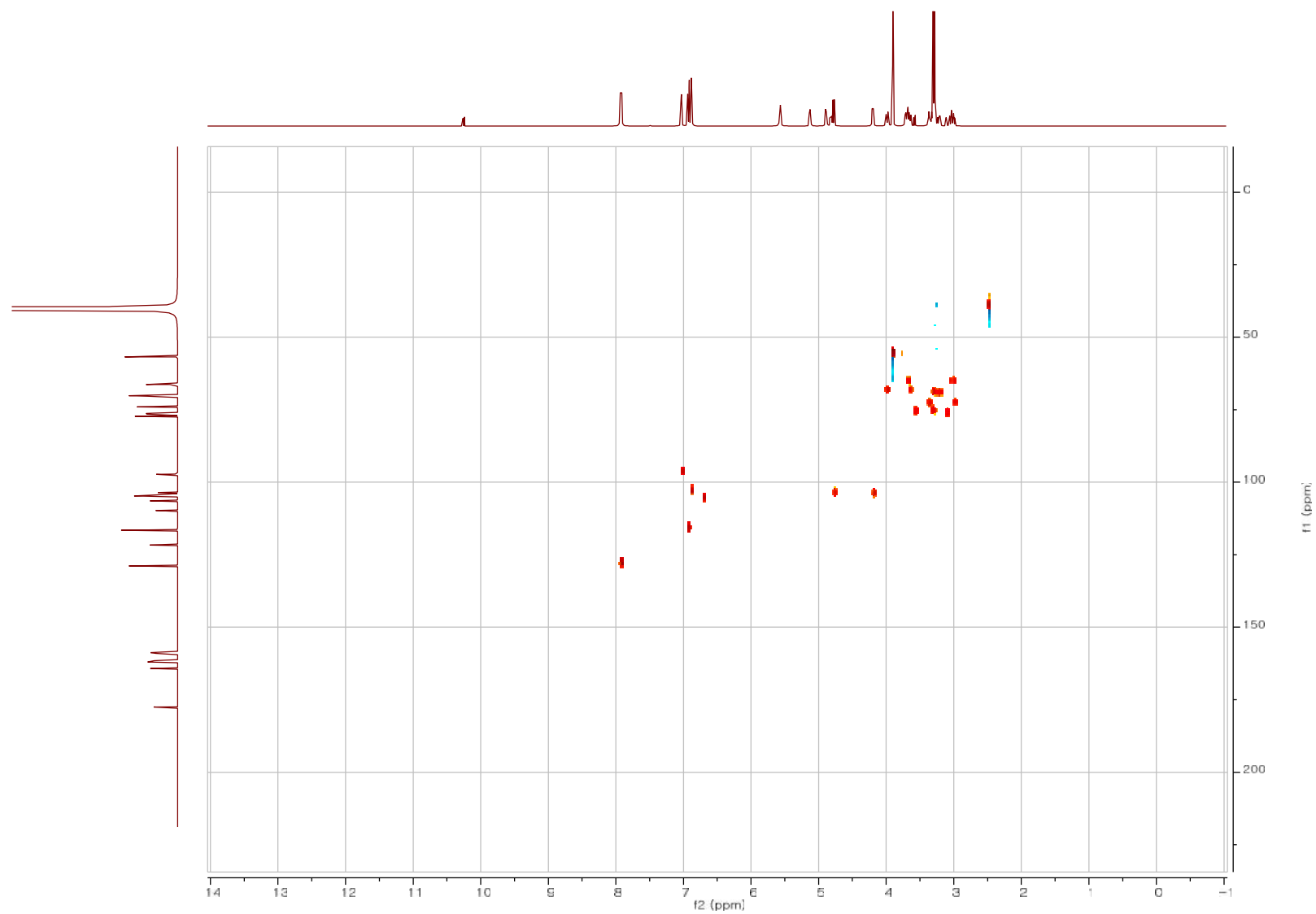
S3. The ^1H - ^1H COSY spectrum of compound 4



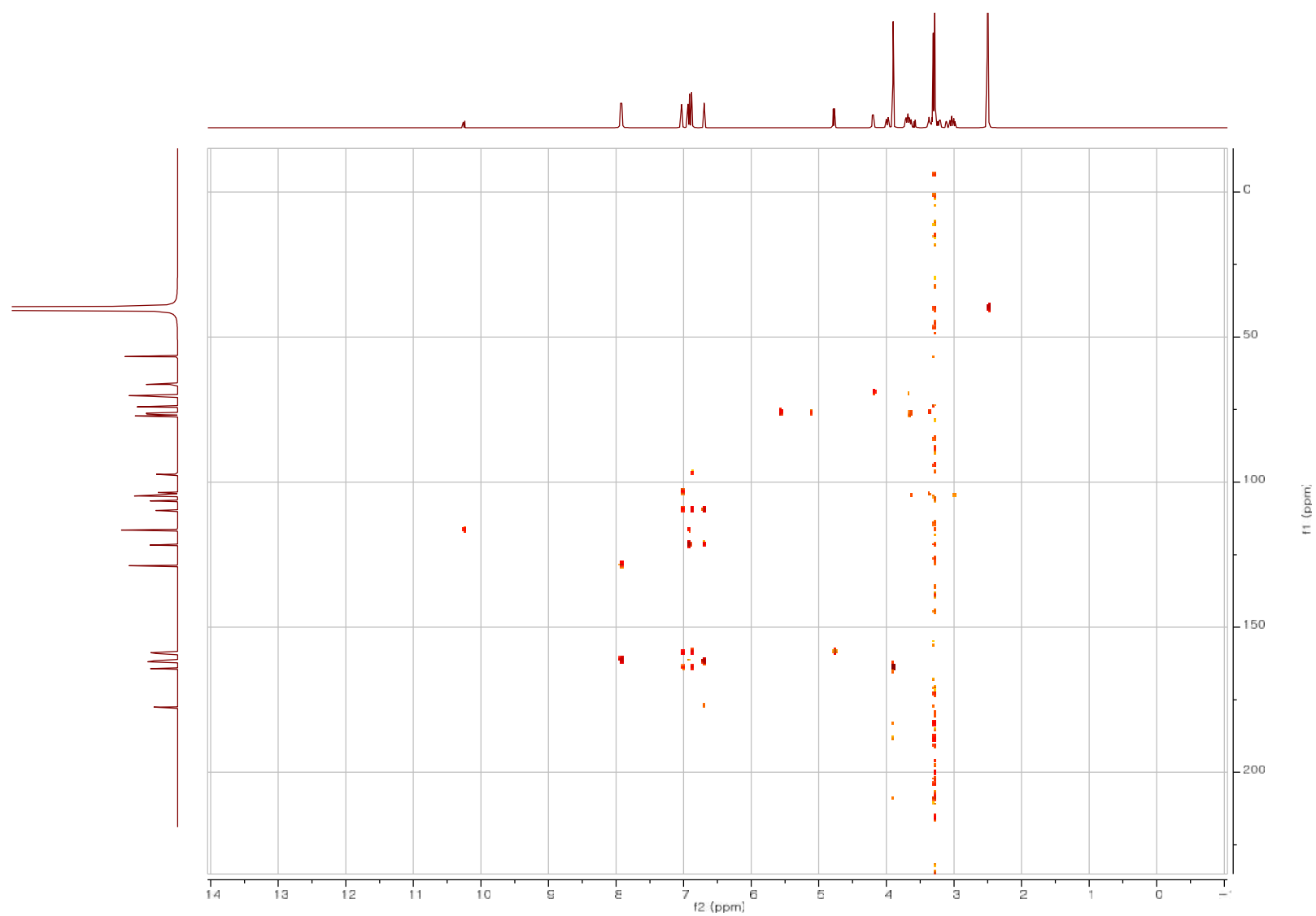
S4. The ^1H - ^1H NOESY spectrum of compound **9**



S5. The HMQC spectrum of compound **4**



S6. The HMBC spectrum of compound **4**



S7. The mass spectrum of compound 4

