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A New Vomifoliol Derivative and Flavonoids from the Aerial Parts of *Orthosiphon aristatus*

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Abstract – *Orthosipon aristatus* (Lamiaceae) is a perennial herbaceous plant native to tropical Southeast Asia countries. The chemical investigation on this plant led to the isolation and structure elucidation of a new vomifoliol derivative (**1**) along with eighteen known phenolic compounds [cystosiphonin (**2**), 5,7,8-trimethoxyflavanone (**3**), scutellarein-7,4′-methylether (**4**), eupatorin (**5**), 5,6,7,8,4′-pentamethoxyflavone (**6**), 5,6,7,4′-tetramethylscutellarein (**7**), 5,6,7,8,3′,4′-hexamethoxyflavone (**8**), 6-hydroxy-5,7,4′-trimethoxyflavone (**9**), 6,7,8,3′,4′-pentamethoxyflavone (**10**), 5,6,3′-trihydroxy-7,4′-dimethoxyflavone (**11**), 5,6-dihydroxy-7,3′,4′-trimethoxy flavone (**12**), 4′-hydroxy-5,6,7,3′-tetramethoxyflavone (**13**), 2′-hydroxy-3,4,4′,5′,6′-pentamethoxy-chalcone (**14**), isoquercetin (**15**), quercetin 3-*O*-*α*-L-arabinopyranosyl (1→6)-*β*-D-glucopyranoside (**16**), caffeic acid (**17**), rosmarinic acid (**18**), and (6*S*,9*R*)-roseoside (**19**)]. The structures of isolates were confirmed *via* spectroscopic data such as one- and two-dimensional nuclear magnetic resonance, circular dichroism, mass, ultraviolet and optical rotation.

Keywords – *Orthosipon aristatus*, **Lamiaceae, Vomifoliol derivative, Phenolic compounds**

Introduction

Orthosiphon aristatus (Blume) Miq. (also known as *Orthosiphon stamineus* Benth.) is a perennial herbaceous shrub that belongs to the Lamiaceae family.[1] It is native to Southeast Asian countries such as Indonesia, Malaysia, Myanmar, Thailand, and Vietnam. It has different vernacular names such as “Kumis Kucing” (Indonesia), “Misai Kucing” (Malaysia), both meaning cat’s whiskers, and “Java tea (Europe)”.

For centuries, *O. aristatus* has been traditionally used as a diuretic due to high level of potassium and for the treatment of arthritis, diabetes, gout, hypertension, renal and urinary disorders, and many scientific studies have supported the rationale behind such traditional uses through anti-inflammatory, anti-oxidative, diuretic, antihypertensive, hypoglycemic and hepatoprotective effects.[2–4] Previous chemical studies demonstrated that *O. aristatus* contained methoxylated flavones, flavonol glycosides, caffeic acid and its derivatives, oxygenated diterpenens, and such flavone and diterpene derivatives have been demonstrated to exert the aforementioned biological activities of *O. aristatus*.[2–4] Among the constituents, eupatorine (3’,5-Dihydroxy-4’,6,7-trimethoxyflavone) and rosmarinic acid were mainly found from the leaves of *O. aristatus* and regarded as biologically active and marker compounds of this plant.[5–6]

As part of ongoing research on medicinal plants of Myanmar origin, we conducted a further chemical investigation on the aerial parts of *O. aristatus*, isolating and elucidating 19 compounds including a new vomifoliol derivative (**1**) and known 2 flavanones (**2**–**3**), 10 flavones (**4**–**13**), a chalcone (**14**), 2 flavonol glycosides (**15**–**16**), caffeic acid derivatives (**17**–**18**) and a norisoprenoid (**19**). The chemical structures of isolates were determined by sp ectroscopic data including one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR), mass (MS), optical rotation, and circular dichroism (CD) spectra.

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Experimental

General experimental Procedures – Ultraviolet (UV) spectra were recorded using a UV-1800 spectrometer (Shimadzu, Japan). Molecular formula was determined by a 6530 ESI-Q-TOF-MS instrument (Agilent Technologies, Santa Clara, CA, USA). 1D- and 2D-NMR experiments were performed by an Ascend TM 500 spectrometer (Bruker, Germany). Optical rotation was measured by a P-2000 polarimeter (Jasco, Tokyo, Japan). A J-815 CD spectrometer (Jasco, Tokyo, Japan) was utilized to record circular dichroism (CD) spectrum. Preparative high-performance liquid chromatography (HPLC) was conducted using a Gilson preparative HPLC system (Gilson, Middelton, WI, USA) comprising binary pumps, a UV/Vis-155 detector and a GX-271 liquid handler and Luna® C18 (2) column (21.2 × 250 mm I.D., 5 mm; Phenomenex, USA). Medium pressure liquid chromatography was performed using Spot II Flash (Interchim, Montluçon, France). Organic solvents for column chromatography were of analytical grade and provided by Daejung Chemical and Metals (Gyeonggi-do, Korea). Thin layer chromatography plate and authentic D-glucose was purchased from Merck (Kenilworth, NJ, USA). HPLC-grade solvents, including methanol, acetonitrile and water, were purchased from JT baker (Center valley, PA, USA). Silica gel 60 and RP-C18 resin (Merck, Kenilworth, NJ, USA), Sephadex LH-20 (Pharmacia Co., Stockholm, Sweden) and ZEOprep 90 C18 (40-63 μm, Zeochme, Uetikon, Switzerland) were used for column chromatography.

Sample materials – The arial parts of *O. aristatus* were collected from Popa Mountain National Park in August 2013, and identified by Khin Myo Htwe (Popa Mountain National Park). The voucher specimen was deposited in the Herbarium of College of Pharmacy, The Catholic University of Korea (#PopaOrothsiphon_A 082013)

Extraction and isolation – The dried leaves of *O.aristatus* (280 g) were extracted with methanol (2.5 L × 3 h × 3 times) using an ultrasonic bath, and the solvent was evaporated under reduced pressure to give 38 g of crude methanol extract. The extract was suspended in water and sequentially partitioned with *n*-hexane, ethyl acetate and *n*-butanol. Ethyl acetate soluble extract (6.6 g) was chromatographed on a silica gel column chromatography (CC) using a gradient elution of *n*-hexane-ethyl acetate (5:1 → 1:1, v/v) followed by chloroform-methanol (5:1 → 1:1, v/v) to yield three subfractions (OE1–OE3). Fraction OE1 was subjected to Sephadex LH-20 CC using 100% methanol to give two subfractions (OE1.1 and OE1.2). OE1.1 was separated by a reversed-phased medium pressure column chromatography (RP-MPLC) with a gradient elution of methanol-water mixture (7:3 → 8:2, v/v) to yield OE1.1.1–OE1.1.4. Compound **2** (1.5 mg) and compound **14** (4.0 mg) were isolated from OE1.1.1 by RP-HPLC using 70% aqueous methanol. Fraction OE2 was chromatographed on a preparative RP-HPLC using 70% aqueous methanol to yield compounds **4** (6.0 mg), **3** (11.0 mg), **5** (18.0 mg), **6** (14.0 mg). Fraction OE2 was resolved by Sephadex LH-20 CC using a methanol as an eluent giving 5 subfractions (OE2.1 – OE2.5). Fraction OE2.4 was further separated by RP-HPLC (70% → 80% aqueous methanol) to give compounds **7** (8.0 mg) and **8** (8.0 mg). Fraction OE3 was subjected to silica gel CC with a gradient elution of chloroform-methanol mixture (25:1 → 5:1, v/v) to yield subfractions OE3.1–OE3.6. OE3.3 was resolved RP-MPLC to give five subfractions (OE3.3.1–OE3.3.5). OE3.3.1 was purified by RP-HPLC with 45% aqueous methanol yielding compound 11(5.0 mg). Compounds **9** (4.0 mg) and **10** (10.0 mg) were separated from fraction OE3.3.2 by RP-HPLC with 50% aqueous methanol as an eluent. Compound **12** (1.5 mg) was purified from fraction OE3.3.4 by RP-HPLC with gradient elution of aqueous methanol (20% → 40%). Compound **13** (3.0 mg) was isolated by RP-HPLC (eluent, 50% aqueous methanol) from OE3.3.5. Fraction OE3.6 was chromatographed on a silica gel CC with gradient elution of chloroform-methanol mixture (5:1 → 1:1, v/v) to yield four subfractions (OE3.6.1–OE3.6.4), and RP-HPLC (50% → 70% aqueous methanol) was used to isolate compounds **1** (3.0 mg), **15** (4.0 mg) and **18** (9.0 mg) from OE3.6.2.

The *n*-butanol soluble extract was separated by silica gel CC with gradient elution of chloroform-methanol mixture (10:1 → 2:1, v/v) to give five subfraction (OB1–OB5). Fraction OB2 was purified through RP-HPLC (50% → 80%, aqueous methanol) to yield compounds **17** (4.0 mg) and **19** (7.0 mg). Compound **16** was isolated from fraction OB4 utilizing RP-HPLC with gradient elution of aqueous methanol (45% → 80%).

Sugar analysis – Compound **1** (1.0 mg) was dissolved in 1.0 mL of 1 N HCl and incubated at 80°C for 2 h. The reaction mixture was neutralized by Ag_2CO_3 followed by the evaporation under nitrogen gas flow, then the residue

was partitioned with deionized water (1.0 mL) and ethyl acetate (1.0 mL). The water layer was analyzed by normal-phase thin layer chromatography using dichloromethane-methanol mixture (10:1, v/v) with aniline phthalate reagent as coloring reagent. The retardation factor of the hydrolysate was identical to that of authentic sugar.

(6*S*,9*R*)-Vomifoliol 9-*O*-(6′-*O*-caffeoyl)-*β*-D-glucopyranoside (1) – brown amorphous powder, $[\alpha]^{25}_{D}$ +27.4° (*c* 0.1, MeOH); UV λ_{max} 232, 298, 329 nm; ESI-QTOF-MS: *m/z* 547.2198 $[M-H]^-$ (calcd. for $C_{28}H_{35}O_{11}$ 547.2179); CD (MeOH) [*θ*] (nm): +31,634 (239), 0 (299), 589 (305); ^{1}H-NMR (CD_3OD and DMSO-d_6, 500MHz): Table 1; ^{13}C-NMR (CD_3OD and DMSO-d_6,125MHz): (Table 1).

Cystosiphonin (2) – yellow amorphous powder; ESI-QTOF-MS *m/z* 383.1122 $[M+Na]^+$ (calcd for $C_{19}H_{20}O_7Na$ 383.1107); ^{1}H-NMR (500 MHz, CD_3OD): *δ* 2.79 (1H, dd, *J*=17.2, 3.0 Hz, H-3), 3.19 (1H, m, H-3), 3.75 (3H, s, 6-OMe), 3.86 (3H, s, 3′-OMe), 3.86 (3H, s, 4′-OMe), 3.88 (3H, s, 7-OMe), 5.43 (1H, dd, *J*=13.0, 2.9 Hz, H-2), 6.24 (1H, s, H-8), 6.98 (1H, d, *J*=8.3 Hz, H-5′), 7.05 (1H, dd, *J*=8.3, 1.9 Hz, H-6′), 7.13 (1H, d, *J*=1.9 Hz, H-2′); ^{13}C-NMR (125 MHz, CD_3OD): *δ* 44.3 (C-3), 56.6 (4′-OMe), 56.7 (3′-OMe), 56.9 (7-OMe), 61.2 (6-OMe), 81.0 (C-2), 93.1 (C-8), 104.2 (C-10), 111.6 (C-2′), 112.9 (C-5′), 120.4 (C-6′), 131.5 (C-6), 133.0 (C-1′), 150.8 (C-3′), 151.0 (C-4′), 156.0 (C-5), 160.7 (C-9), 162.5 (C-7), 198.8 (C-4).

5,7,8-Trimethoxyflavanone (3) – yellow amorphous powder; ESI-QTOF-MS *m/z* 337.1065 $[M+Na]^+$ (calcd for $C_{18}H_{18}O_5Na$ 337.1052); ^{1}H-NMR (500 MHz, CD_3OD): *δ* 2.79 (1H, dd, *J*=16.7, 3.1 Hz, H-3), 3.02 (1H, dd, *J*=16.7, 12.5 Hz, H-3), 3.73 (3H, s, 8-OMe), 3.88 (3H, s, 5-OMe), 3.95 (3H, s, 7-OMe), 5.49 (1H, dd, *J*=12.5, 3.0 Hz, H-2), 6.33 (1H, s, H-6), 7.37 (1H, d, *J*=7.3 Hz, H-4′), 7.42 (2H, t, *J*=7.3 Hz, H-3′ and H-5′), 7.52 (2H, d, *J*=7.3 Hz, H-2′ and H-6′); ^{13}C-NMR (125 MHz, CD_3OD): *δ* 46.5 (C-3), 56.5 (5-OMe), 56.8 (7-OMe), 61.5 (8-OMe), 80.5 (C-2), 90.9 (C-6), 106.9 (C-10), 127.4 (C-2′ and C-6′), 129.7 (C-3′ and C-5′), 129.8 (C-4′), 132.1 (C-8), 140.6 (C-1′), 157.8 (C-9),

Table 1. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectroscopic data of compound **1**.

	CD_3OD		DMSO-d_6	
No	^{1}H-NMR (multiplicity, *J* in Hz)	^{13}C-NMR	^{1}H-NMR (multiplicity, *J* in Hz)	^{13}C-NMR
1		42.6		40.9
2	2.47 (1H, d, 16.8), 2.13 (1H, d, 16.8)	50.9	2.47 (1H, d, 16.8), 2.03 (1H, d, 16.8)	49.3
3		201.4		197.3
4	5.85 (1H, s)	127.3	5.76 (1H, s)	125.6
5		167.3		166.5
6		80.1		77.8
7	5.84 (1H)*	131.8	5.72 (1H)*	130.4
8	5.84 (1H)*	135.0	5.71 (1H)*	132.8
9	4.39 (1H)**	77.2	4.30 (1H)**	74.5
10	1.30 (1H, d, 5.9)	21.3	1.19 (1H, d, 6.4)	20.8
11	1.88 (1H, d, 1.1)	19.8	1.88 (1H, d, 1.4)	18.9
12	1.01 (3H, s)	23.6	0.91 (3H, s)	23.0
13	1.00 (3H, s)	24.8	0.89 (3H, s)	23.0
1′	4.39 (1H)**	103.2	4.26 (1H, d, 7.8)	101.1
2′	3.20 (1H)***	75.3	2.97 (1H, brt, 8.2)	73.7
3′	3.36 (1H)***	78.1	3.15 (1H)***	76.5
4′	3.36 (1H)***	71.7	3.15 (1H)***	69.8
5′	3.47 (1H)***	75.6	3.34 (1H)***	73.6
6′	4.45 (1H, dd, 11.9, 1.9), 4.30 (1H, dd, 11.9, 5.3)	64.7	4.31 (1H)**, 4.13 (dd, 11.8, 5.8)	63.4
1″		127.8		125.5
2″	7.05 (1H, d, 1.6)	114.9	7.05 (1H, d, 2.2)	115.0
3″		147.3		145.5
4″		149.8		148.3
5″	6.78 (1H, d, 8.1)	115.7	6.76 (1H, d, 8.1)	115.7
6″	6.96 (1H, dd, 8.1, 1.6)	121.2	7.00 (1H, dd, 8.1, 2.2)	121.2
7″	7.56 (1H, d, 15.9)	146.9	7.46 (1H, d, 15.9)	145.1
8″	6.29 (1H, d, 15.9)	113.8	6.27 (1H, d, 15.9)	113.9
9″		166.5		163.9

*, **, *** peaks overlapped by each other or solvent or impurity peak; The assignments were based on ^{1}H-^{1}H COSY, HSQC, and HMBC experiments.

159.7 (C-5), 161.0 (C-7), 192.1 (C-4).

Scutellarein-7,4′-methylether (**4**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 337.0693 $[M+Na]^+$ (calcd for $C_{17}H_{14}O_6Na$ 337.0688); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.86 (3H, s, 7-OMe), 3.92 (3H, s, 6-OMe), 6.90 (1H,s, H-3), 6.94 (1H, s, H-8), 7.12 (2H, d, *J*=8.7 Hz, H-3′ and H-5′), 8.07 (2H, d, *J*=8.7 Hz, H-2′ and H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 55.5 (7-OMe), 56.3 (6-OMe), 91.2 (C-8), 103.1 (C-3), 105.1 (C-10), 114.6 (C-3′ and C-5′), 123.0 (C-1′), 128.2 (C-2′ and C-6′), 130.0 (C-6), 146.2 (C-9), 149.6 (C-5), 154.4 (C-7), 162.9 (C-4′), 163.3 (C-2), 182.2 (C-4).

Eupatorin (**5**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 367.0808 $[M+Na]^+$ (calcd for $C_{18}H_{16}O_7Na$ 367.0794); ^{1}H-NMR (500 MHz, CD_3OD): δ 3.73 (3H, s, 6-OMe), 3.87 (3H, s, 4′-OMe), 3.92 (3H, s, 7-OMe), 6.80 (1H,s, H-3), 6.89 (1H, s, H-8), 7.08 (1H, d, *J*=8.3 Hz, H-5′), 7.48 (1H, d, *J*=1.9 Hz, H-2′), 7.56 (1H, dd, *J*=8.3, 1.9 Hz, H-6′); ^{13}C-NMR (125 MHz, CD_3OD): δ 55.7 (4′-OMe), 56.4 (7-OMe), 60.0 (6-OMe), 91.5 (C-8), 103.3 (C-3), 105.1 (C-10), 112.0 (C-5′), 113.0 (C-2′), 118.6 (C-6′), 122.9 (C-1′), 131.9 (C-6), 146.9 (C-3′), 151.2 (C-4′), 152.1 (C-5), 152.6 (C-9), 158.6 (C-7), 163.8 (C-2), 182.1 (C-4).

5,6,7,8,4′-Pentamethoxyflavone (**6**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 395.1121 $[M+Na]^+$ (calcd for $C_{20}H_{20}O_7Na$ 395.1107); ^{1}H-NMR (500 MHz, CD_3OD): δ 3.77 (3H, s, 8-OMe), 3.83 (3H, s, 6-OMe), 3.85 (3H, s 4′-OMe), 3.96 (3H, s, 5-OMe), 4.02 (3H, s, 7-OMe), 6.76 (1H,s, H-3), 7.13 (2H, d, *J*=8.7 Hz, H-3′ and H-5′), 7.99 (2H, d, *J*=8.7 Hz, H-2′ and H-6′); ^{13}C-NMR (125 MHz, CD_3OD): δ 55.5 (4′-OMe), 61.4 (8-OMe), 61.5 (6-OMe), 61.8 (7-OMe), 61.9 (5-OMe), 106.0 (C-3), 114.3 (C-10), 114.6 (C-3′ and C-5′), 123.0 (C-1′), 127.7 (C-2′ and C-6′), 137.7 (C-8), 143.5 (C-6), 147.1 (C-9), 147.5 (C-5), 150.9 (C-4′), 160.3 (C-2), 162.0 (C-7), 175.7 (C-4).

5,6,7,4′-Tetramethylscutellarein (**7**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 365.1019 $[M+Na]^+$ (calcd for $C_{19}H_{18}O_6Na$ 365.1001); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.66 (3H, s, 4′-OMe), 3.80 (3H, s, 7-OMe), 3.85 (3H, s, 6-OMe), 3.95 (3H, s, 5-OMe), 6.72 (1H, s, H-8), 7.10 (2H, d, *J*=8.9 Hz, H-3′ and H-5′), 7.22 (1H, s, H-3), 8.02 (2H, d, *J*=8.9 Hz, H-2′ and H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 55.7 (4′-OMe), 56.2 (7-OMe), 60.6 (6-OMe), 61.4 (5-OMe), 96.7 (C-8), 105.8 (C-3), 111.8 (C-10), 114.2 (C-3′ and C-5′), 122.8 (C-1′), 127.3 (C-2′ and C-6′), 139.5 (C-6), 151.2 (C-9), 153.6 (C-5), 157.1 (C-7), 160.2 (C-4′), 161.6 (C-2), 175.4 (C-4).

5,6,7,8,3′,4′-Hexamethoxyflavone (**8**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 425.1232 $[M+Na]^+$ (calcd for $C_{21}H_{22}O_8Na$ 425.1212); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.78 (3H, s, 8-OMe), 3.84 (3H, s, 6-OMe), 3.85 (3H, s, 4′-OMe), 3.88 (3H, s, 3′-OMe), 3.97 (3H, s, 5-OMe), 4.02 (3H, s, 7-OMe), 6.87 (1H, s, H-3), 7.16 (1H, d, *J*=8.5 Hz, H-5′), 7.54 (1H, d, *J*=2.0 Hz, H-2′), 7.65 (1H, dd, *J*=8.5, 2.0 Hz, H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 55.3 (6-OMe), 55.3 (8-OMe), 61.0 (4′-OMe), 61.1 (3′-OMe), 61.4 (7-OMe), 61.5 (5-OMe), 105.9 (C-3), 108.5 (C-2′), 111.5 (C-5′), 113.9 (C-10), 118.9 (C-6′), 122.7 (C-1′), 137.3 (C-6), 143.1 (C-5), 146.8 (C-9), 147.1 (C-8), 148.6 (C-3′), 150.6 (C-7), 151.4 (C-4′), 159.9 (C-2), 175.4 (C-4).

6-Hydroxy-5,7,4′-trimethoxyflavone (**9**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 351.0862 $[M+Na]^+$ (calcd for $C_{18}H_{16}O_6Na$ 351.0845); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.74 (3H, s, 5-OMe), 3.85 (3H, s, 4′-OMe), 3.93 (3H, s, 7-OMe), 6.68 (1H, s, H-3), 7.10 (2H, d, *J*=8.9 Hz, H-3′ and H-5′), 7.15 (1H, s, H-8), 8.01 (2H, d, *J*=8.9 Hz, H-2′ and H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 55.4 (4′-OMe), 56.2 (7-OMe), 61.1 (5-OMe), 96.6 (C-8), 105.9 (C-3), 111.9 (C-10), 114.4 (C-3′ and C-5′), 123.2 (C-1′), 127.6 (C-2′ and C-6′), 137.5 (C-6), 144.2 (C-5), 150.7 (C-9), 153.2 (C-7), 160.0 (C-2), 161.7 (C-4′), 175.7 (C-4).

6,7,8,3′,4′-Pentamethoxyflavone (**10**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 395.1124 $[M+Na]^+$ (calcd for $C_{20}H_{20}O_7Na$ 395.1107); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.77 (3H, s, 8-OMe), 3.80 (3H, s, 6-OMe), 3.85 (3H, s, 4′-OMe), 3.89 (3H, s, 3′-OMe), 3.96 (3H, s, 7-OMe), 6.80 (1H, s, H-3), 7.11 (1H, d, *J*=8.6 Hz, H-5′), 7.22 (1H, s, H-5), 7.55 (1H, d, *J*=2.1 Hz, H-2′), 7.66 (1H, dd, *J*=8.6, 2.1 Hz, H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 55.6 (6-OMe), 55.8 (8-OMe), 56.4 (4′-OMe), 60.9 (3′-OMe), 61.8 (7-OMe), 97.3 (C-5), 106.3 (C-3), 109.1 (C-2′), 111.6 (C-10), 112.0 (C-5′), 119.4 (C-6′), 123.1 (C-1′), 139.7 (C-6), 149 (C-3′), 151.5 (C-8), 151.6 (C-4′), 153.9 (C-9), 157.4 (C-7), 160.2 (C-2), 175.3 (C-4).

5,6,3′-Trihydroxy-7,4′-dimethoxyflavone (**11**) – yellow amorphous powder; ESI-QTOF-MS *m/z* $[M+Na]^+$ (calcd for $C_{17}H_{14}O_7Na$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.87 (3H, s, 4′-OMe), 3.92 (3H, s, 7-OMe), 6.78 (1H, s, H-3), 6.90 (1H, s, H-8), 7.10 (1H, d, *J*=8.6 Hz, H-5′), 7.47 (1H, d, *J*=2.3 Hz, H-2′), 7.57 (1H, dd, *J*=8.6, 2.3 Hz, H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 55.7 (4′-OMe), 56.2 (7-

OMe), 91.1 (C-8), 103.1 (C-3), 105.0 (C-10), 112.1 (C-5′), 113.0 (C-2′), 118.5 (C-6′), 123.1 (C-1′), 129.9 (C-6), 146.1 (C-5), 146.8 (C-3′), 149.6 (C-9), 151.0 (C-4′), 154.3 (C-7), 163.5 (C-2), 182.1 (C-4).

5,6-Dihydroxy-7,3′,4′-trimethoxyflavone (**12**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 343.0824 $[M-H]^-$ (calcd for $C_{18}H_{15}O_7$ 343.0818); ^{1}H-NMR (500 MHz, CD_3OD): δ 3.86 (3H, s, 4′-OMe), 3.90 (3H, s, 3′-OMe), 3.93 (3H, s, 7-OMe), 6.96 (1H, s, H-8), 7.00 (1H, s, H-3), 7.14 (1H, d, *J*=8.6 Hz, H-5′), 7.60 (1H, d, *J*=2.1 Hz, H-2′), 7.72 (1H, d, *J*=8.7, 2.1 Hz, H-6′); ^{13}C-NMR (125 MHz, CD_3OD): δ 55.7 (4′-OMe), 55.9 (3′-OMe), 56.3 (7-OMe), 91.2 (C-8), 103.4 (C-3), 105.0 (C-10), 109.4 (C-2′), 111.7 (C-5′), 119.9 (C-6′), 123.0 (C-1′), 129.9 (C-6), 146.1 (C-5), 149.0 (C-3′), 149.6 (C-9), 152.0 (C-4′), 154.4 (C-7), 163.3 (C-2), 182.2 (C-4).

4′-Hydroxy-5,6,7,3′-tetramethoxyflavone (**13**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 357.0986 $[M-H]^-$ (calcd for $C_{19}H_{18}O_7$ 357.0974); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.76 (3H, s, 6-OMe), 3.80 (3H, s, 5-OMe), 3.90 (3H, s, 3′-OMe), 3.95 (3H, s, 7-OMe), 6.74 (1H, s, H-3), 6.93 (1H, d, *J*=8.8 Hz, H-6′), 7.21 (1H, s, H-8), 7.54 (1H, s, H-2′), 7.55 (1H, dd, *J*=7.2, 1.9 Hz, H-5′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 55.9 (3′-OMe), 56.4 (7-OMe), 60.9 (6-OMe), 61.8 (5-OMe), 97.3 (C-8), 105.8 (C-3), 109.9 (C-2′), 112.0 (C-10), 115.6 (C-5′), 119.7 (C-6′), 121.7 (C-1′), 139.6 (C-6), 147.9 (C-4′), 150.1 (C-3′), 151.5 (C-9), 153.9 (C-5), 157.3 (C-7), 160.6 (C-2), 175.6 (C-4).

2′-Hydroxy-3,4,4′,5′,6′-pentamethoxychalcone (**14**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 397.1279 $[M+Na]^+$ (calcd for $C_{20}H_{22}O_7Na$ 397.1263); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.69 (3H, s, 5′-OMe), 3.81 (3H, s, 3-OMe), 3.82 (3H, s, 4-OMe), 3.83 (3H, s, 4′-OMe), 3.83 (3H, s, 6′-OMe), 6.38 (1H, s, H-3′), 7.02 (1H, d, *J*=8.3 Hz, H-5), 7.29 (1H, d, *J*=8.3, 1.8 Hz, H-6), 7.31 (1H, d, *J*=1.8 Hz, H-2), 7.43 (1H, d, *J*=15.7 Hz, H-8′), 7.55 (1H, d, *J*=15.7 Hz, H-9′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 55.5 (4-OMe), 55.6 (3-OMe), 56.0 (4′-OMe), 60.6 (5′-OMe), 61.4 (6′-OMe), 96.4 (C-3′), 110.7 (C-2), 110.7 (C-1′), 111.7 (C-5), 122.8 (C-6), 125.0 (C-8′), 127.3 (C-1), 134.6 (C-5′), 143.9 (C-9′), 148.9 (C-3), 151.1 (C-4), 152.9 (C-6′), 157.3 (C-2′), 157.6 (C-4′), 192.6 (C-7′).

Isoquercetin (**15**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 463.0889 $[M-H]^-$ (calcd for $C_{21}H_{19}O_{12}$ 463.0877); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.17–3.58 (6H, m, H-2″ and H-6″), 5.46 (1H, d, *J*=7.4 Hz, H-1″), 6.20 (1H, d, *J*=1.6 Hz, H-6), 6.40 (1H, d, *J*=1.6 Hz, H-8), 6.84 (1H, d, *J*=9.0 Hz, H-5′), 7.57 (1H, s, H-2′), 7.58 (1H, dd, *J*=9.0, 2.1 Hz, H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 60.9 (C-6″), 69.9 (C-4″), 74.0 (C-2″), 76.4 (C-3″), 77.5 (C-5″), 93.4 (C-8), 98.6 (C-6), 100.8 (C-1″), 103.9 (C-10), 115.1 (C-5′), 116.1 (C-2′), 121.1 (C-6′), 121.5 (C-1′), 133.2 (C-3), 144.7 (C-3′), 148.4 (C-4′), 156.1 (C-9), 156.2 (C-2), 161.2 (C-5), 164.0 (C-7), 177.4 (C-4).

Quercetin 3-*O*-*α*-L-arabinopyranosyl (1→6)-*β*-D-glucopyranoside (**16**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 595.1311 $[M-H]^-$ (calcd for $C_{26}H_{28}O_{16}$ 595.1299); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 2.90 (1H, d, *J*=10.4 Hz, H-5‴), 3.00–3.43 (3H, m, H-2‴ and H-4‴), 3.10–3.30 (4H, m, H-2″ and H-5″), 3.44 (1H, m, H-6″), 3.47 (1H, dd, *J*=12.1, 2.9 Hz, H-5‴), 3.77 (1H, d, *J*=10.6 Hz, H-6″), 3.95 (1H, d, *J*=7.0 Hz, H-1‴), 5.37 (1H, d, *J*=7.4 Hz, H-1″), 6.19 (1H, d, *J*=2.0 Hz, H-6), 6.38 (1H, d, *J*=2.0 Hz, H-8), 6.85 (1H, d, *J*=9.0 Hz, H-5′), 7.58 (1H, d, *J*=2.0 Hz, H-2′), 7.58 (1H, d, *J*=9.0, 2.0 Hz, H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 64.7 (C-5‴), 67.2 (C-6″), 67.2 (C-4‴), 70.0 (C-4″), 70.4 (C-2‴), 72.4 (C-3‴), 73.8 (C-2″), 76.2 (C-3″), 76.8 (C-5″), 93.4 (C-8), 98.6 (C-6), 100.7 (C-1″), 102.6 (C-1‴), 103.9 (C-10), 115.2 (C-5′), 116.1 (C-2′), 121.0 (C-1′), 121.5 (C-6′), 133.2 (C-3), 144.7 (C-3′), 148.4 (C-4′), 156.2 (C-2), 156.3 (C-9), 161.2 (C-5), 164.1 (C-7), 177.3 (C-4).

Caffeic acid (**17**) – yellow amorphous powder; ESI-QTOF-MS *m/z* 179.0347 $[M-H]^-$ (calcd for $C_9H_8O_4$ 179.0344); ^{1}H-NMR (500 MHz, CD_3OD): δ 6.22 (1H, d, *J*=15.8 Hz, H-8), 6.78 (1H, d, *J*=8.1 Hz, H-5), 6.93 (1H, d, *J*=8.1 Hz, H-6), 7.03 (1H, s, H-2), 7.53 (1H, d, *J*=15.8 Hz, H-7); ^{13}C-NMR (125 MHz, CD_3OD): δ 113.8 (C-8), 115.2 (C-2), 116.6 (C-5), 122.9 (C-6), 128.0 (C-1), 146.7 (C-3), 146.9 (C-7), 149.4 (C-4), 171.8 (C-9).

Rosmarinic acid (**18**) – brown amorphous powder; ESI-QTOF-MS *m/z* 359.0783 $[M-H]^-$ (calcd for $C_{18}H_{16}O_8$ 359.0767); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 2.90 (1H, dd, *J*=14.3, 8.6 Hz, H-11a), 2.99 (1H, dd, *J*=14.3, 3.8 Hz, H-11b), 5.02 (1H, dd, *J*=8.5, 4.0 Hz, H-10), 6.24 (1H, d, *J*=16.1 Hz, H-8), 6.52 (1H, dd, *J*=8.0, 1.5 Hz, H-17), 6.64 (1H, d, *J*=8.0 Hz, H-16), 6.68 (1H, br, H-13), 6.77 (1H, d, *J*=8.1 Hz, H-5), 7.01 (1H, d, *J*=8.1 Hz, H-6), 7.05 (1H, br, H-2), 7.46 (1H, d, *J*=16.1 Hz, H-7); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 36.1 (C-11), 72.8 (C-10), 113.3 (C-8), 114.8 (C-2), 115.4 (C-16), 115.8 (C-5), 116.7 (C-13), 120.1 (C-17), 121.7 (C-6), 125.4 (C-1), 127.3 (C-12), 144.0 (C-

3), 144.9 (C-4), 145.5 (C-14), 145.9 (C-7), 148.6 (C-15), 166.0 (C-9), 170.9 (C-18).

(6*S*,9*R*)-Roseoside (**19**) – brown amorphous powder; ESI-QTOF-MS *m/z* 431.1919 $[M+HCOO]^-$ (calcd for $C_{20}H_{31}O_{10}$ 431.1917); ^{1}H-NMR (500 MHz, CD_3OD): δ 1.03 (3H, s, H-12), 1.04 (3H, s, H-13), 1.29 (3H, d, *J*=6.4 Hz, H-10), 1.92 (3H, d, *J*=1.3 Hz, H-11), 2.15 (1H, d, *J*=17.0 Hz, H-2), 2.52 (1H, d, *J*=17.0 Hz, H-2), 3.17 (1H, dd, *J*=9.2, 7.8 Hz, H-2′), 3.22 (1H, m, H-5′), 3.25 (1H, m, H-4′), 3.33 (1H, m, H-3′), 3.62 (1H, dd, *J*=11.7, 5.5 Hz, H-6′), 3.85 (1H, dd, *J*=11.8, 2.0 Hz, H-6′), 4.34 (1H, d, *J*=7.8 Hz, H-1′), 4.42 (1H, m, H-9), 5.85 (1H, s, H-4), 5.87 (2H, m, H-7 and H-8), ^{13}C-NMR (125 MHz, CD_3OD): δ 19.7 (C-11), 21.3 (C-10), 23.5 (C-12), 24.8 (C-13), 42.5 (C-1), 50.8 (C-2), 62.9 (C-6′), 71.8 (C-4′), 75.3 (C-2′), 77.4 (C-9), 78.1 (C-5′), 78.2 (C-3′), 80.1 (C-6), 102.8 (C-1′), 127.3 (C-4), 131.6 (C-7), 135.4 (C-8), 167.4 (C-5), 201.3 (C-3).

Results and Discussion

Chemical investigation of ethyl acetate and *n*-butanol soluble extracts of aerial parts of *O. aristatus* led to the isolation and structure elucidation of 19 compounds including a new compound **1** and **18** known ones (**2**–**19**) (Fig. 1).

Compound **1** was isolated as brown amorphous powder, and its molecular formula was determined to be $C_{28}H_{36}O_{11}$ from the pseudomolecular ion peak at *m/z* 547.2198 $[M-H]^-$ by ESI-QTOF-MS. The ^{1}H-NMR spectrum (in methanol-d_4) of **1** showed characteristic signals for a caffeoyl moiety [1,3,4-trisubstituted benzene ring at δ_H 7.05 (1H, d, *J*=1.6 Hz, H-2″), 6.96 (1H, dd, *J*=8.1, 1.6 Hz, H-6″) and 6.78 (1H, d, *J*=8.1 Hz, H-5″), a pair of olefinic resonances at δ_H 7.56 (1H, d, *J*=15.9 Hz, H-7″) and 6.29 (1H, d, *J*=15.9 Hz, H-8″)]. Besides a caffeoyl moiety, the

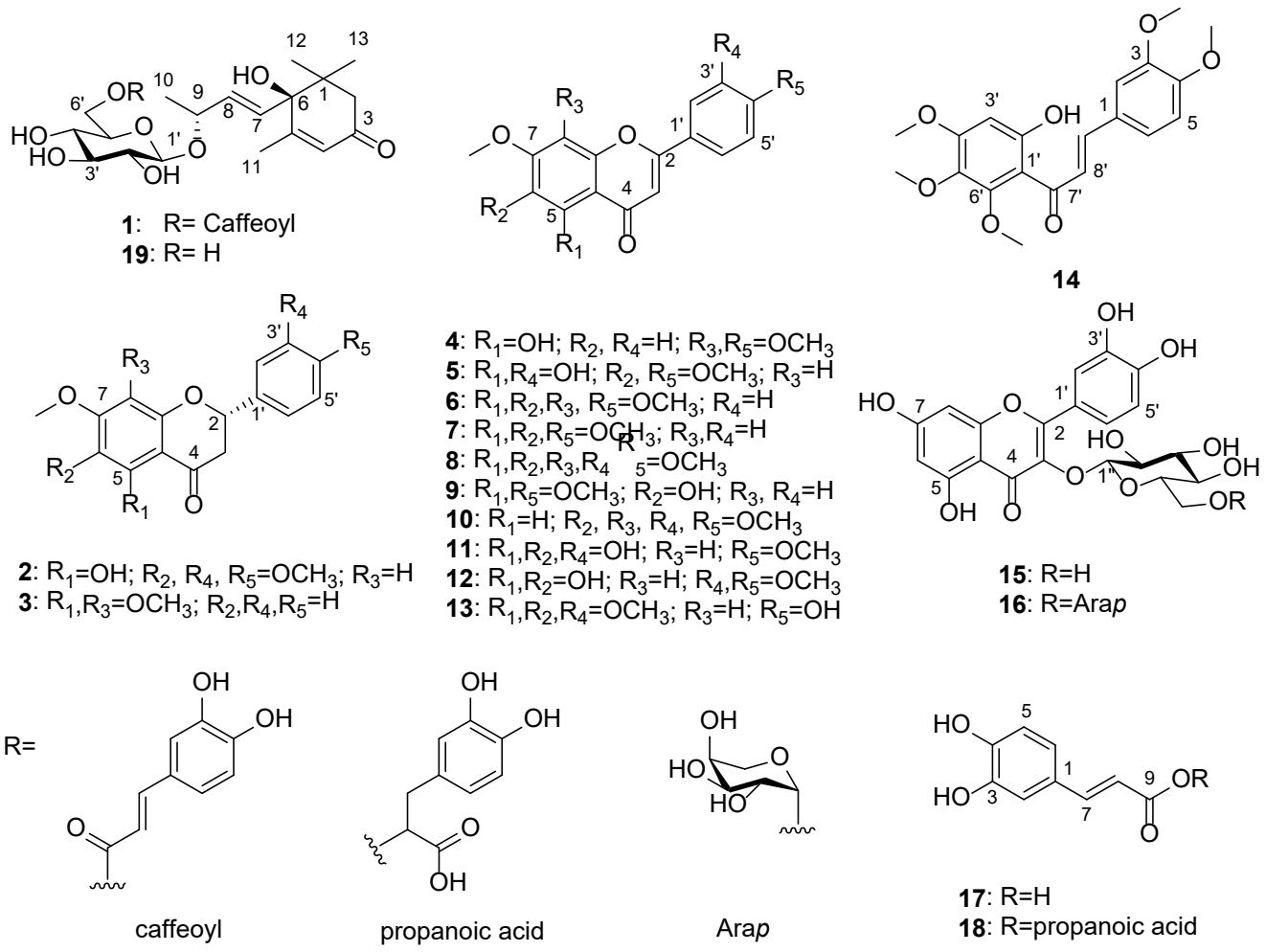

Fig 1. Chemical structures of isolated compounds 1−19.

combination of 1D- and 2D-NMR experiments exhibited the structural information of a vomifoliol scaffold [four methyl groups [δ_H 1.88 (1H, d, *J*=1.1 Hz and H-11)/δ_C 19.8; δ_H 1.30 (1H, d, *J*=5.9 Hz, H-10)/δ_C 21.3; δ_H 1.01 (3H, s, H-12)/δ_C 23.6; δ_H 1.00 (3H, s, H-13)/δ_C 24.8), three methines [δ_H 5.84 (1H, peak overlapped, H-7)/δ_C 135.0; δ_H 5.84(1H, overlapped peak, H-7)/δ_C 131.8; δ_H 5.85 (1H, s, H-4)/δ_C 127.3)], an oxygenated methine [δ_H 4.39 (1H, peak overlapped, H-9)/δ_C 77.2], a methylene [δ_H 2.47 (1H, d, *J*=16.8 H and H-2a) and 2.13 (1H, d, *J*=16.8 Hz, H-2b)/δ_C 50.9)], three quaternary carbons [δ_C 167.3 (C-5), 80.1 (C-6) and 42.6 (C-1), a ketone [δ_C 201.4 (C-3)], and an anomeric proton and carbon resonances of a sugar moiety [δ_H 4.39 (1H, peak overlapped, H-1″)/δ_C 103.2] (Table 1). The sugar moiety was confirmed to be *β*-D-glucopyranoside by acid hydrolysis and ^{1}H and ^{13}C NMR spectrum. The connectivity of three functional groups (caffeoyl, vomifoliol and *β*-D-glucopyranose moieties) was confirmed by HMBC experiment showing cross peaks from δ_H 4.45 (H-6′b) and 4.30 (H-6′a) to δ_C 166.5 (C-9″) and δ_H 4.39 (H-1′) to δ_C 77.2 (C-9) (Fig. 2). The ^{1}H- and ^{13}C-NMR were further recorded in DMSO-d_6 to clarify the structure of **1** (Table 1). Finally, the absolute configuration of C-6 and C-9 positions was determined to be 6*S*,9*R* through the positive Cotton effect at 239 nm in circular dichroism spectrum and the chemical shift of C-9 [δ_C 77.2 ppm (in case of 9*S*: δ_C ~74.7 ppm)], respectively.[7] Therefore, the chemical structure of **1** was established to be (6*S*,9*R*)-vomifoliol 9-*O*-(6′-*O*-caffeoyl)-*β*-D-glucopyranoside.

The eighteen known compounds were identified to be cystosiphonin (**2**),[8] 5,7,8-trimethoxyflavanone (**3**),[9] scutellarein-7,4′-methylether (**4**),[10] eupatorin (**5**),[11] 5,6,7,8,4′-pentamethoxyflavone (**6**),[12] 5,6,7,4′-tetramethylscutellarein (**7**),[13,14] 5,6,7,8,3′,4′-hexamethoxyflavone (**8**),[12] 6-hydroxy-5,7,4′-trimethoxyflavone (**9**),[15] 6,7,8,3′,4′-pentamethoxyflavone (**10**),[12] 5,6,3′-trihydroxy-7,4′-dimethoxyflavone (**11**),[16] 5,6-dihydroxy-7,3′,4′-trimethoxyflavone (**12**),[16] 4′-hydroxy-5,6,7,3′-tetramethoxyflavone (**13**),[14] 2′-hydroxy-3,4,4′,5′,6′-pentamethoxy-chalcone (**14**),[17] isoquercetin (**15**),[18] quercetin 3-*O*-*α*-L-arabinopyranosyl (1 → 6)-*β*-D-glucopyranoside (**16**),[19,20] caffeic acid (**17**),[21] rosmarinic acid (**18**),[22] and (6*S*,9*R*)-roseoside (**19**)[7] by comparison of their spectroscopic data with those of reported values. To the best of our knowledge, compounds **8** and **14** were found in the Lamiaceae family for the first time in this study. The presence of **11**–**13** and the other compounds have been determined in the Lamiaceae family and *O. aristatus,* respectively.

Acknowledgments

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

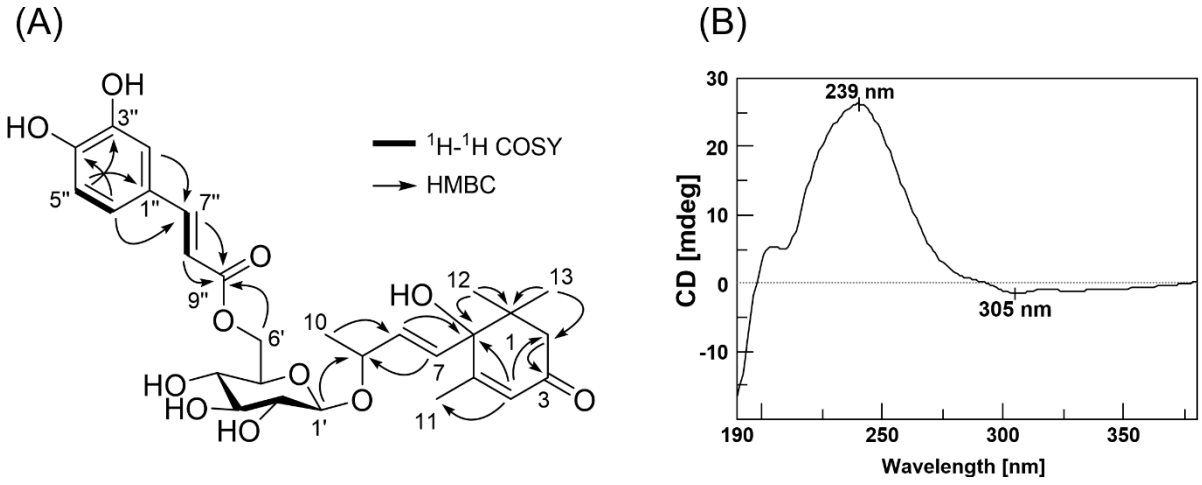

Fig 2. ^{1}H-^{1}H COSY and HMBC correlations (A) and CD spectrum (B) of **1**.

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Supplementary Information

A New Vomifoliol Derivative and Flavonoids from the Aerial Parts of *Orthosiphon aristatus*

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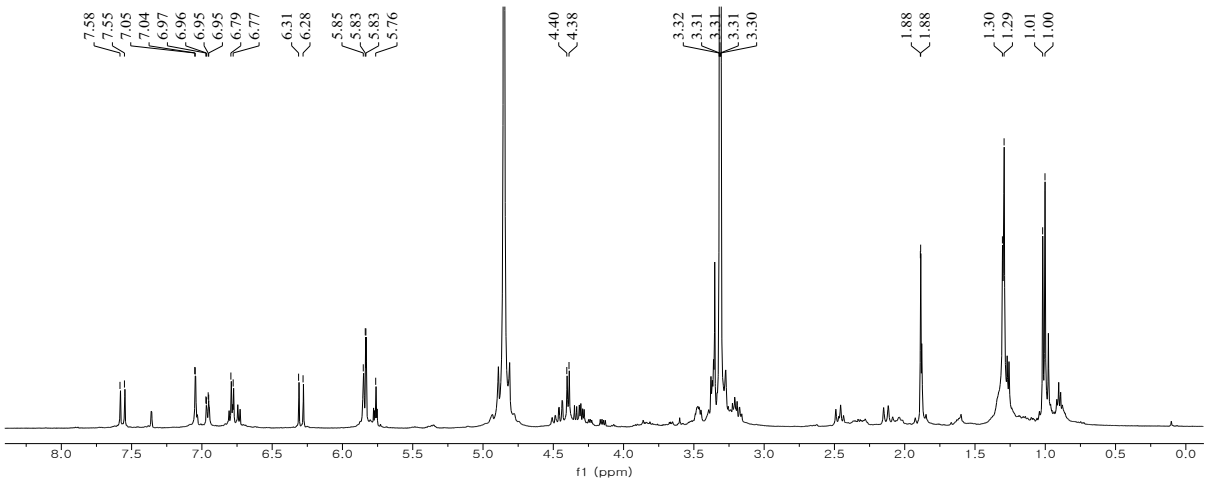

Figure S1. 1H-NMR spectrum of compound **1** (CD3OD, 500 MHz).

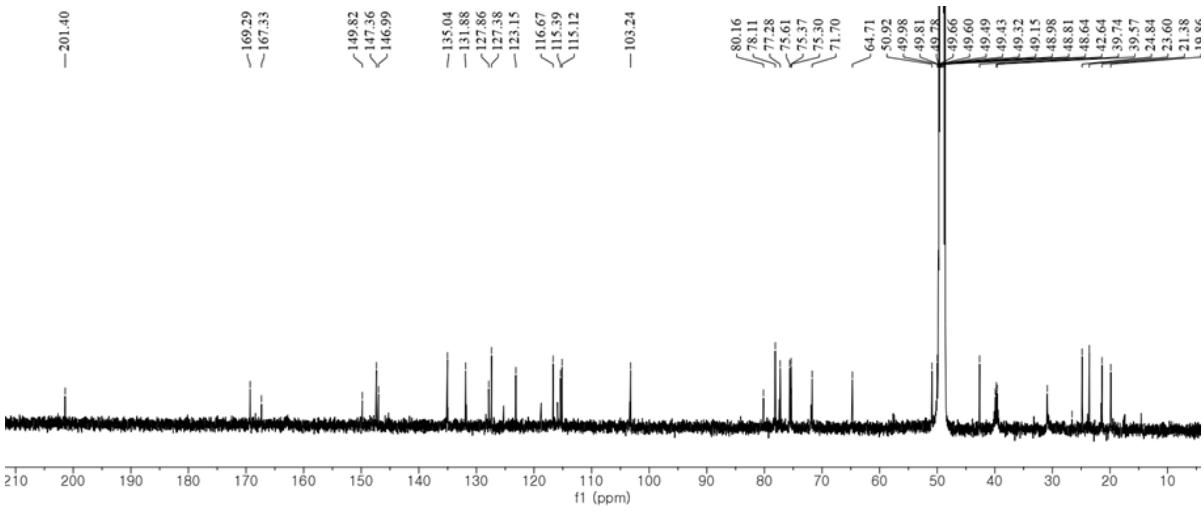

Figure S2. 13C-NMR spectrum of compound **1** (CD3OD, 125MHz).

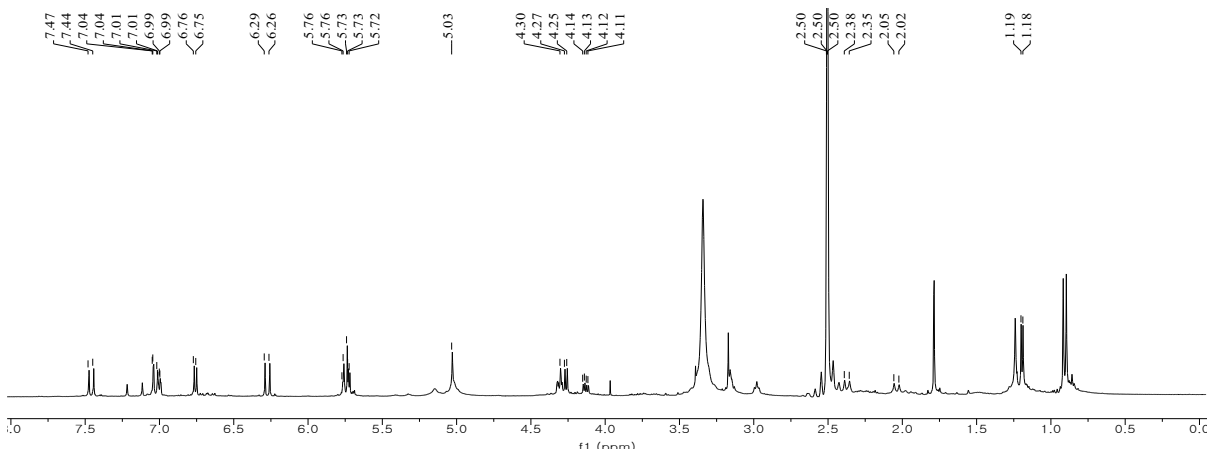

Figure S3. 1H-NMR spectrum of compound **1** (DMSO-d6, 500 MHz).

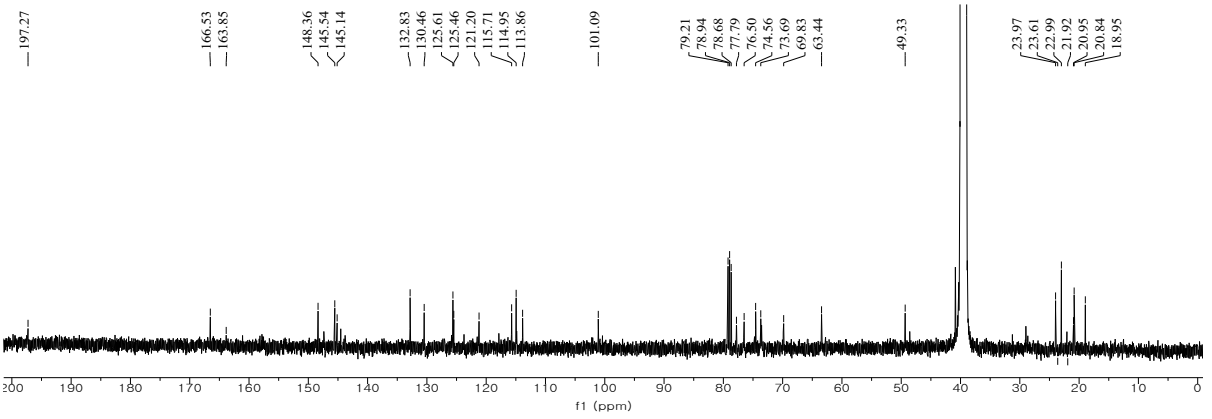

Figure S4. 13C-NMR spectrum of compound **1** (DMSO-d6, 125MHz).

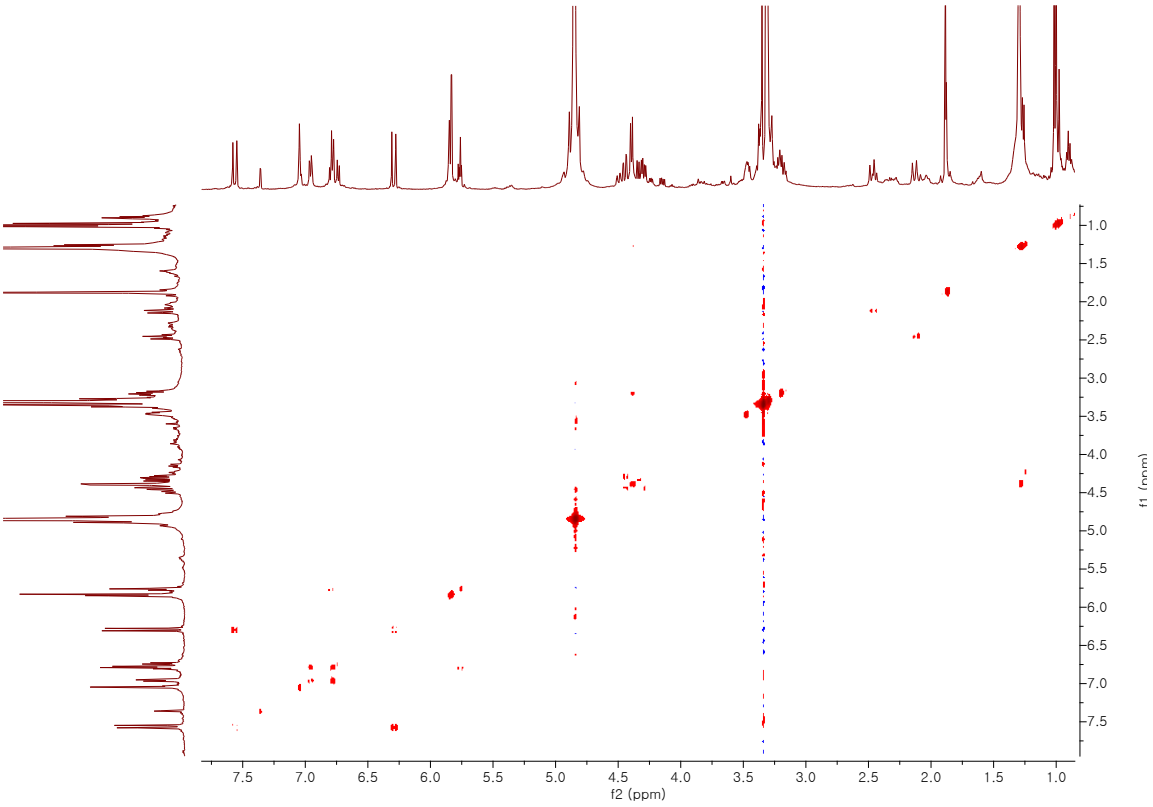

Figure S5. 1H-1H COSY spectrum of compound **1** (CD3OD).

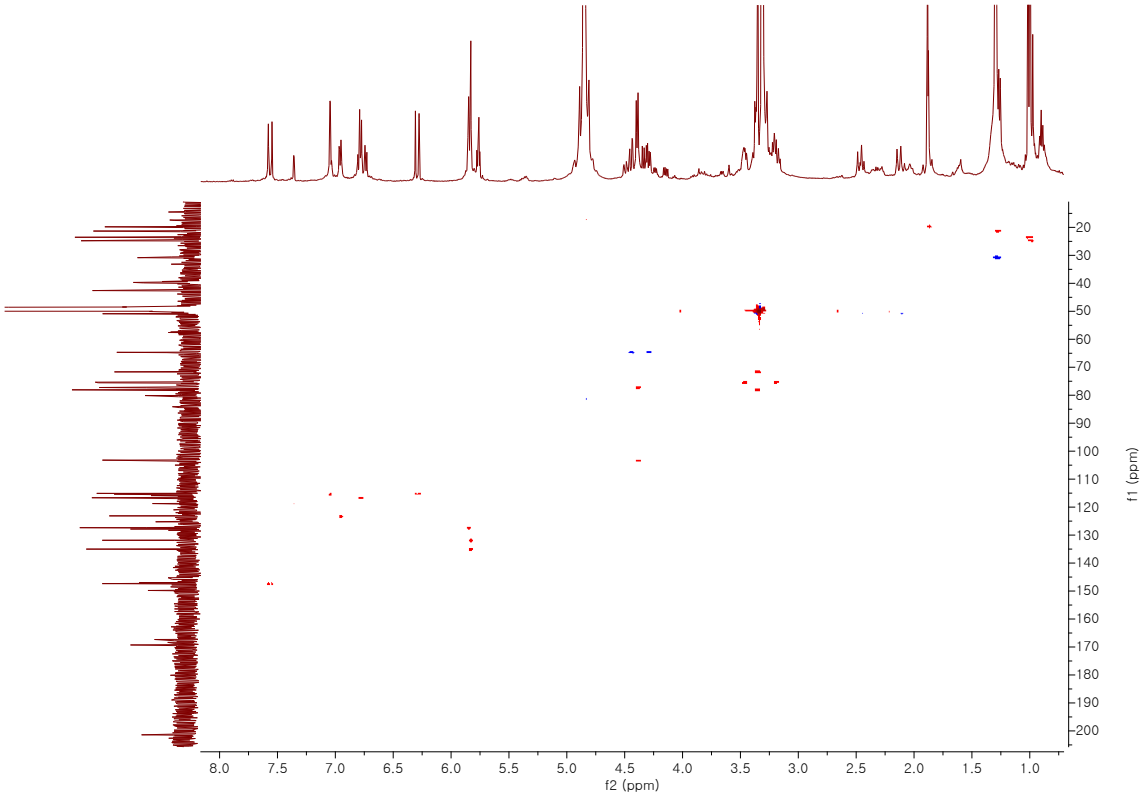

Figure S6. HSQC spectrum of compound **1** (CD3OD).

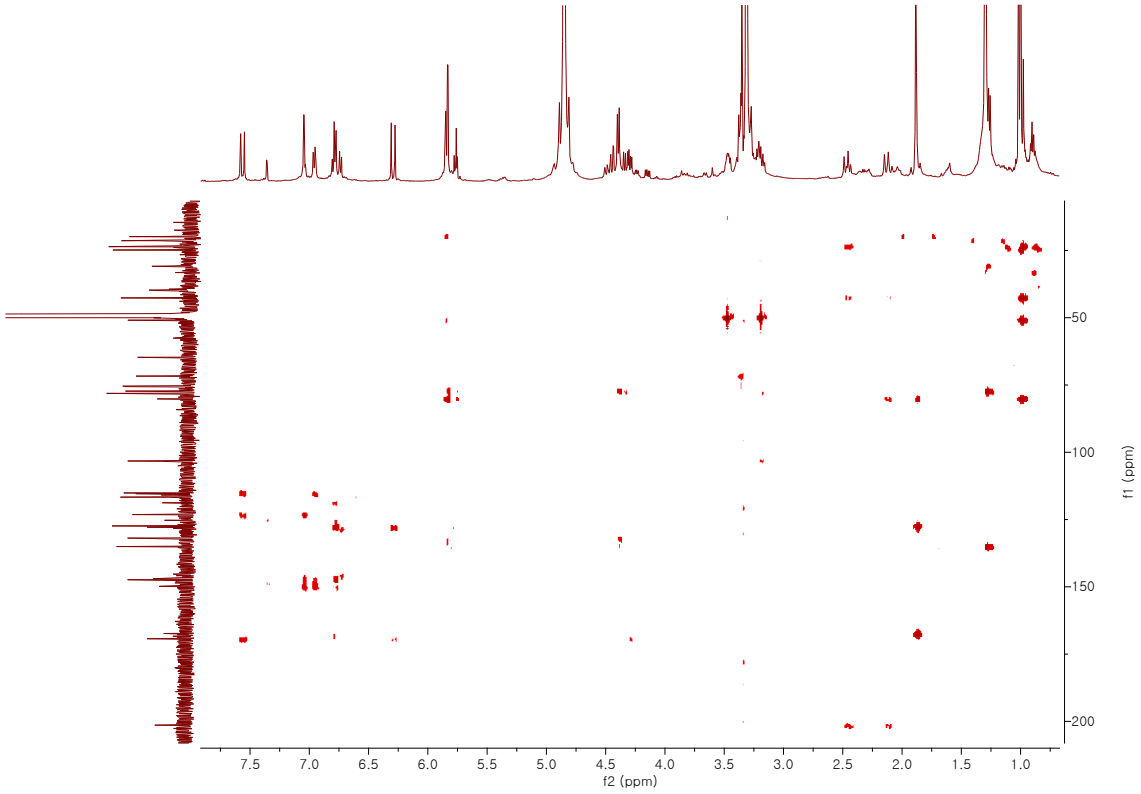

Figure S7. HMBC spectrum of compound **1** (CD3OD).

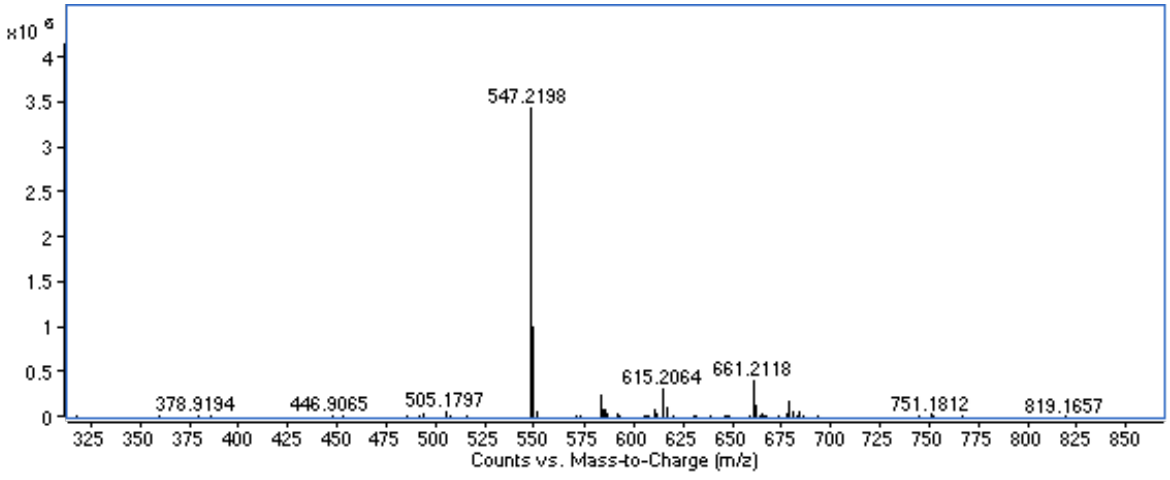

Figure S8. ESI-Q-TOF MS spectrum of compound **1**