Supporting Information

Composition decipherment of *Ficus pumila* var. *awkeotsang* and its potential on COVID-19 symptom amelioration and *in silico* prediction of SARS-CoV-2 interference.

Hao-Chun Hu[1,†], Szu-Yin Yu[2,†], Xiao-Shan Hung[1], Chun-Han Su[3,4], Yu-Liang Yang[3,5], Chien-Kei Wei[1], Yuan-Bin Cheng[6], Yang-Chang Wu[6,7,8], Chia-Hung Yen[1], Tsong-Long Hwang[9,10,11], Shu-Li Chen[1], István Szatmári [12], Attila Hunyadi[2], Yi-Hong Tsai[1,13,*], Fang-Rong Chang[1,6,14,15,*]

[1] Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 80708, Taiwan.
[2] Institute of Pharmacognosy, Interdisciplinary Excellence, Centre University of Szeged, Szeged, Hungary
[3]Agricultural Biotechnology Research Center, Academia Sinica, Taipei 115, Taiwan
[4] Research Center for Chinese Herbal Medicine, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan 333, Taiwan.
[5] Biotechnology Center in Southern Taiwan, Academia Sinica, Tainan 711, Taiwan
[6] Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan.
[7] Graduate Institute of Integrated Medicine, China Medical University, Taichung 40402, Taiwan
[8] Chinese Medicine Research and Development Center, China Medical University Hospital, Taichung 40402, Taiwan
[9] Graduate Institute of Natural Products, College of Medicine, Chang Gung University, Taoyuan 33302, Taiwan.
[10] Research Center for Chinese Herbal Medicine, Research Center for Food and Cosmetic Safety, and Graduate Institute of Health Industry Technology, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan 33303, Taiwan.
[11] Department of Anesthesiology, Chang Gung Memorial Hospital 33305 Taoyuan, Taiwan.
[12] Institute of Pharmaceutical Chemistry and ELKH-MTA-SZTE Stereochemistry Research Group, Hungarian Academy of Sciences, University of Szeged, Szeged, Hungary
[13]Department of Pharmacy and Master Program, Collage of Pharmacy and Health Care, Tajen University, Pingtung County 90741, Taiwan.
[14] Drug Development and Value Creation Research Center, Kaohsiung Medical University, Kaohsiung 80708, Taiwan
[15]Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

[†] These authors contributed equally

This article is dedicated to the memory of Prof. Dr. Ferenc Fülöp

* Corresponding authors. E-mail addresses: aaronfrc@kmu.edu.tw (F. R. Chang), lyph0719@hotmail.com (Y. H. Tsai)

Contents

1. Protocols of bioassays and preliminary data

In vitro evaluation of anti-inflammatory activity

The assessment of anti-inflammatory activity was conducted using human neutrophils. Blood was withdrawn from healthy volunteers (20–35 years old) following a protocol approved by the institutional review board at Chang Gung Memorial Hospital adopting a standard method. The inhibition of superoxide generation was assessed with the assay based on the reduction of ferricytochrome *c*. Meanwhile, the release of elastase from activated neutrophils was measured using the elastase substrate, *N*-methoxysuccinyl-Ala-Pro-Val-*p*-nitroanilide, following the previously described procedure (*Front Pharmacol* 2020;11:572009).

Assays of Superoxide Anion Generation and Elastase Release

The assays of superoxide anion generation and elastase release in response to fMLF stimulation by neutrophils were conducted with the same method described in the article, *Front Pharmacol* 2020;11:572009, published by one of the current co-authors, Prof. Tsong-Long Hwang.

Cell Culture and Luciferase Reporter Assay

HacaT, immortalized human keratinocytes, were cultured in the Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) with penicillin (100 U/mL), streptomycin (100 g/mL) and 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT, USA) in a humidified incubator (5% CO_2). HacaT/ARE stable cells were grown in DMEM supplemented with hygromycin (100 g/mL). HacaT/ARE stable cells were seeded (1×10^4 cells/well) in 96 well plates overnight. After this, cells were incubated with the test compounds for 18 h. At the assay time point, resazurin (Cayman Chemical, Ann Arbor, MI, USA) was added to a final concentration of 0.1 mg/mL, and the cells were further incubated for 4 h at 37°C. Fluorescence of the reduced resazurin (ex/em: 530 nm/590 nm) was measured from the culture supernatant by using a Synergy HT Multi-Mode Reader (BioTek, Winooski, VT, USA) to determine cell viability. Then the cells were harvested for luciferase activity measurements according to the manufacturer's protocol (Promega Corporation, Madison, WI, USA). Relative luciferase activity was calculated by normalizing luciferase activity to cell viability. DMSO solvent control was used as 100% activity.

For evaluating the cell viability, cells were seeded in 96-well plates containing 100μL per well culture medium overnight. The cells were incubated with the compounds in triplicates for 72 h. At the assay time point, resazurin (Cayman Chemical) was added and the cells were further incubated for 4 h at 37°C. Fluorescence of the reduced resazurin was measured as described above.

Assay of Nrf2 Activation

The assay of Nrf2 activation assays were carried out with the same method as published in *Molecules* 2020;25:3133 by one of the co-authors, Dr. Chia-Hung Yen.

Cytotoxicity assay on cancer cell lines

The method for cytotoxicity assay was performed as previously described (Hu et al. 2020). Briefly, three human cancer cell lines, HepG2 (1×10^4 cells), A549 (5×10^3 cells), and MDA-MB-231 (1×10^4 cells), were inoculated onto 96-well plates and treated with the samples (20 μg/mL). The medium was removed after 72 h of incubation, then 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (100 μL, 0.5 mg/mL) was added into each well. Then, the plates were incubated at 37 °C for 1 h. The MTT dye was detected by the addition of 100 μL of dimethyl sulfoxide. The absorbance was read at 550 nm. The positive control was doxorubicin.

Table S1. Preliminary screens of bioactivities of FPATM fractions - anti-neutrophilic inflammation

	Inhibition (%)	
	Superoxide anion	Elastase release
FPATM-h	71.87 ± 7.10 ***	63.66 ± 4.80 ***
FPATM-75	95.25 ± 5.16 ***	70.91 ± 7.72 ***
FPATM-b	39.81 ± 5.83 **	21.48 ± 2.06 ***

Percentage of inhibition (Inh %) at 10 μg/mL concentration. Results are presented as mean±S.E.M. (n=3~4). *P<0.05, ** P<0.01; *** P<0.001 compared with the control (DMSO).

Table S2. Preliminary screens of bioactivities on FPATM fractions – Nrf2 activation

	(%, mean±SD)[a] Nrf2 activation in HacaT cells
FPATM-h	230 ± 40.0
FPATM-75	620 ± 48.0
FPATM-b	87 ± 3.0
tBHC[b]	592.4 ± 58.4

[a]HaCaT, a normal skin cell line. Drug concentration was 100 μg/mL. [b]tBQC, 2-(1,1-Dimethylerhyl)-1,4-benzenediol, was used as positive control for Nrf2 activation. Drug concentration was 10 μM.

Table S3. Preliminary screens of bioactivities of FPATM fractions – cytotoxicity, and the cell viability of HaCaT cells treated with pure compounds

	Inhibition (%)			Cell Viability (%)
	HepG2	MDA-MB-231	A-549	HaCaT[c]
FPATM-h	13.1	24.1	17.2	
FPATM-75	-46.5	13.2	-45.5	
FPATM-b	-27.3	3.7	-44.2	
Doxorubincin[a]	70.3	82.6	83.0	
1				87.0 ± 0.2
2				97.1 ± 2.8
3				101.8 ± 2.3
4				99.7 ± 0.4
5				99.3 ± 0.5
6				100.7 ± 2.6
7				100.8 ± 3.0
8				99.7 ± 2.7
9				98.6 ± 1.2
10				93.8 ± 5.9
11				97.5 ± 2.3
12				90.1 ± 1.6
13				87.1 ± 0.7
14				96.9 ± 1.8
15				99.5 ± 2.2
16				104.2 ± 1.9
17				94.0 ± 4.1
18				87.1 ± 1.7
19				99.3 ±1.1
20				100.3 ± 2.0
21				87.2 ± 1.2
22				89.4 ± 3.2
23				88.5 ± 1.9
24				93.5 ± 0.5
25				87.8 ± 0.8
26				89.0 ± 3.3
27				92.1 ± 1.1
28				87.3 ± 0.9
tBHQ[b]				105.0 ± 5.3

[a] Positive control: Doxorubicin (IC_{50}, 1μg/mL); FPATM: Methanol extract of twigs from *Ficus pumila* var. *awkeotsang*; FPATM-h: hexane layer of FPATM; FPATM-b: *n*-Butanol layer of FPATM; FPATM-75: 75% MeOH/H_2O (1:1) layer from FPATM

[b] tBHQ, 2-(1,1-Dimethylethyl)-1,4-benzenediol, was used as positive control for Nrf2 activation. The drug concentration is 10 μM.

[c] HaCaT, a normal skin cell line. The drug concentration is 10 μM.

2. Extraction and Isolation

The air-dried twigs (5.5 kg) of FPA (FPAT) were extracted with 95% MeOH at room temperature. By using reduced pressure concentration, 307.0 g crude extract (FPATM) was yielded. FPATM was suspended in H_2O and partitioned with EtOAc to afford two portions. H_2O was partitioned with *n*-Butanol (FPATM-b). Subsequently, the EtOAc layer was partitioned with *n*-hexane (FPATM-h) and 75% MeOH/H_2O (1:1) (FPATM-75). The FPATM-75 (38.0 g) was subjected to a silica gel column chromatography and stepwise eluted with *n*-hexane/EtOAc to yield six fractions. (Fr. A−F). Fr. D (1.0 g) was further passed through Sephadex® LH-20 (Fine Chemicals AB, Uppsala, Pharmacia) in an environment of MeOH−CH_2Cl_2 (1:1) to give four subfractions. NP-CN column (Luna phenyl-hexyl, 100 Å, 250 × 10 mm, Phenomenex®) was then utilized and isocratically eluted with *n*-hexane/EtOAc (15:1) to isolate seventeen compounds, i.e., alloxanthoxyletin (**2**, 6.4 mg), xanthyletin (**3**, 0.9 mg), luvangetin (**4**, 1.0 mg), trachyphyllin (**5**, 0.5 mg), coumarin (**6**, 1.9 mg), limettin (**7**, 0.5 mg), 5,6,7-trimethoxycoumarin (**8**, 1.0 mg), demethylsuberosin (**9**, 0.6 mg), osthole (**10**, 1.9 mg), sibiricol (**11**, 3.6 mg), pinnarin (**12**, 2.3 mg), angelicin (**14**, 1.0 mg), bergapten (**15**, 13.3 mg), xanthotoxin (**16**, 1.9 mg), imperatorin (**17**, 0.4 mg), spathelichromen (**20**, 14.0 mg) and betulinic acid (**24**, 90.6 mg). (*Z*)-3-(3-(2-hydroxypropan-2-yl)-2,3-dihydro-[1,4]dioxino[2,3-g]benzo-furan-5-yl)acrylic acid (**19**, 5.9 mg) was obtained from Fr. E with silica gel column eluted by CH_2Cl_2−EtOAc−MeOH, 70:1:0−0:0:1, and purified by NP-HPLC (*n*-Hexane/EtOAc, 3:1; flow rate: 2 ml/min ; Luna CN (5μm 100 Å 250 × 10 mm, Phenomenex®). Fr. F (7.0 g) was chromatographed over a silica gel column to give five subfractions (Fr. F1−F5). Ficumarin (**1**, 36.7 mg), 5-methoxymarmesin (**13**, 4.2 mg), and ficuformodiol A (21, 2.8 mg) were obtained from Fr. F1 (58.4 mg) isolated by NP-CN column (*n*-Hexane/EtOAc, 7:3). Fr. F4 (2.3 g) was further subjected to another silica gel column (CH_2Cl_2−Acetone–MeOH, 5:1:0−0:0:1), and NP-CN column (*n*-hexane/EtOAc, 5:1) to afford six compounds, i.e., oxypeucedanin hydrate (**18**, 345.8 mg), vomifoliol (**22**, 106.8 mg), dehydrovomifoliol (**23**, 380.2 mg), taxifolin (**25**, 2.9 mg), catechin (**26**, 70.0 mg), epiphyllocoumarin (**27**, 4.6 mg), and vanillic acid (**28**, 1.0 mg).

3. Physical data of compound 1

Ficumarin (**1**): colorless oil; $[\alpha]_D^{22}$ –82 (c 0.05, MeOH); UV (MeOH) λmax (log ε) 216 (3.85), 284 (3.56), 321 (3.58) nm; IR (neat) vmax：3354 cm^{-1}, 1633 cm^{-1}; The ^1H NMR data showed the present of one methyl singlet [δH 1.41 (3H, s)], five olefinic methines [δH 5.53 (^1H , d, J = 10.1 Hz), 6.14 (^1H , d, J = 9.6 Hz), 6.33 (^1H , s), 6.77 (^1H , d, J = 10.1 Hz), and 7.92 (^1H , d, J = 9.6 Hz)], one methylene [δH 3.68 (^1H , d, J = 11.9 Hz), 3.72 (^1H , d, J = 11.9 Hz)], and one methoxy [δH 3.86 (3H, s)] (Table 1). The ^{13}C data of 1 indicated the presence of fifteen carbons signals, including one ester carbonyl (δC 161.5), five non protonated sp^2 carbons (δC 103.6, 106.4, 150.0, 156.1, 158.5), five olefinic methines (δC 92.0, 111.6, 118.8, 123.7, 138.4), one oxygen-bearing quaternary carbon (δC 81.1), one oxygenated methylene (δC 68.8), one methoxy (δC 56.1), and one methyl (δC 23.0).; HRESIMS m/z 297.07336 $[M+Na]^+$ (calcd for $C_{15}H_{14}O_5$ Na^+: 297.07334).

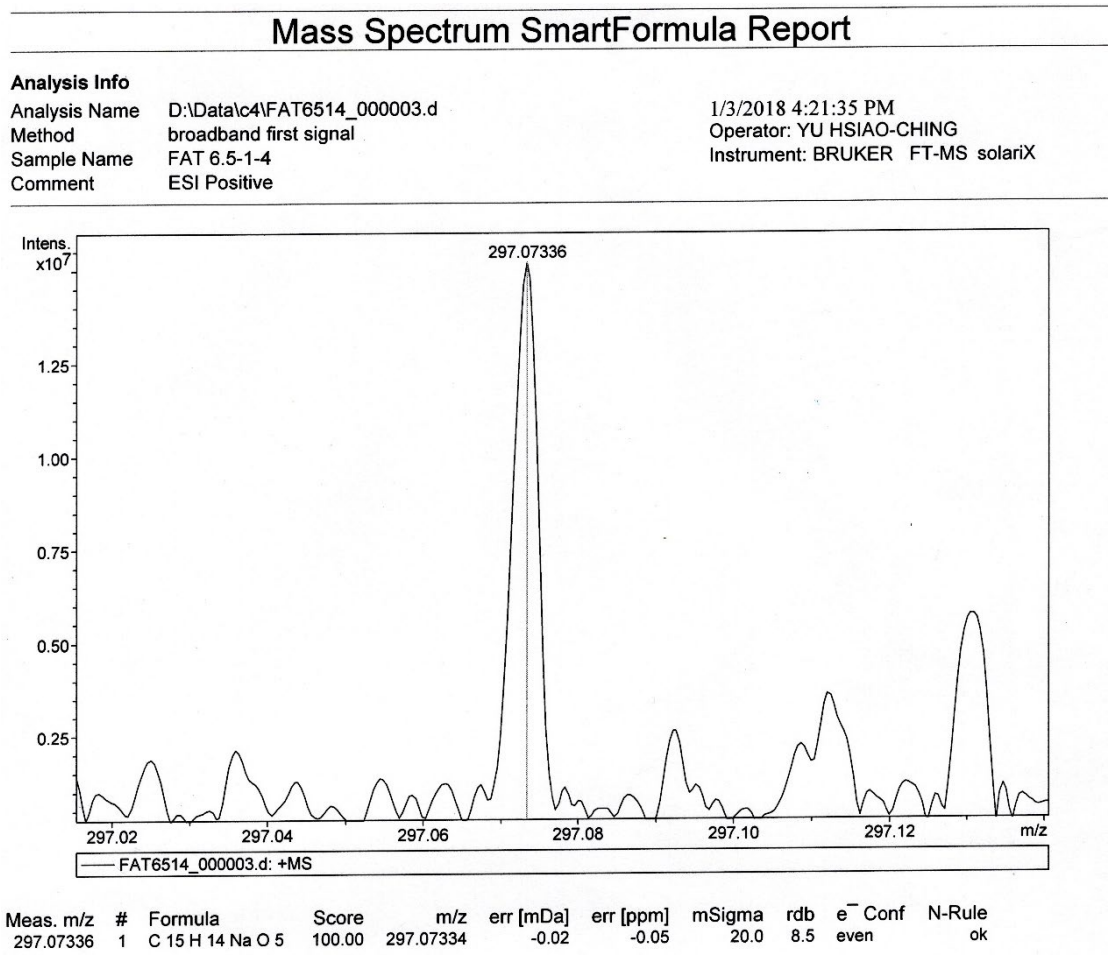

Figure S1. HRESI-MS of **1**

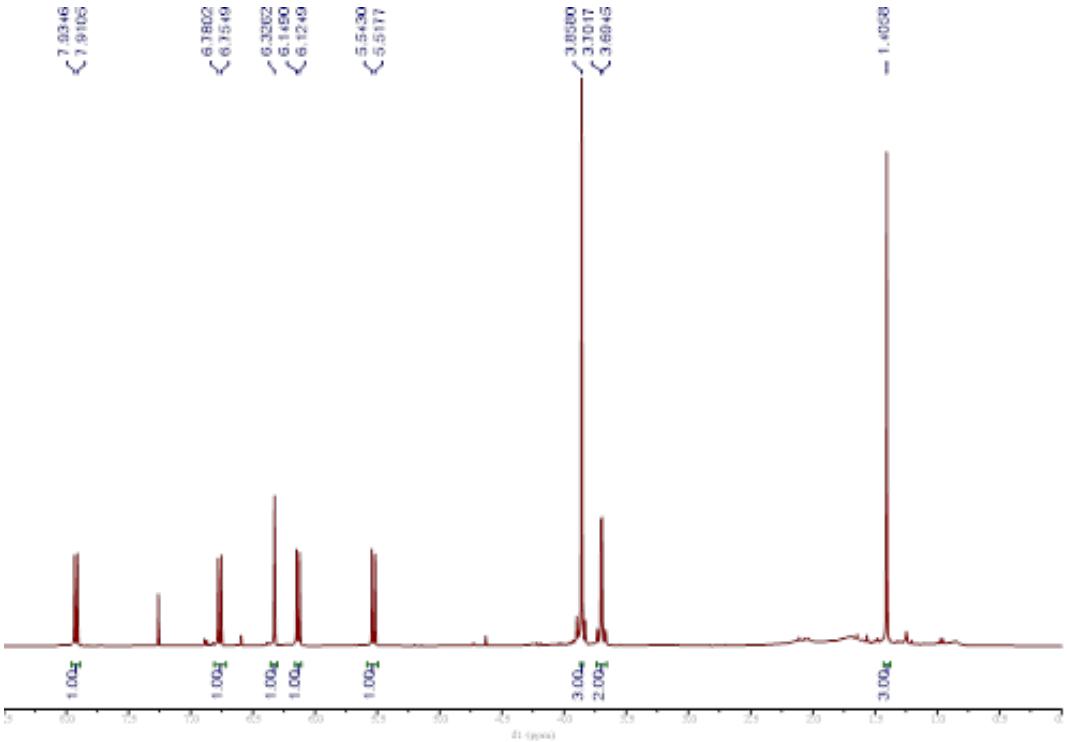

Figure S2. ^{1}H NMR of **1**

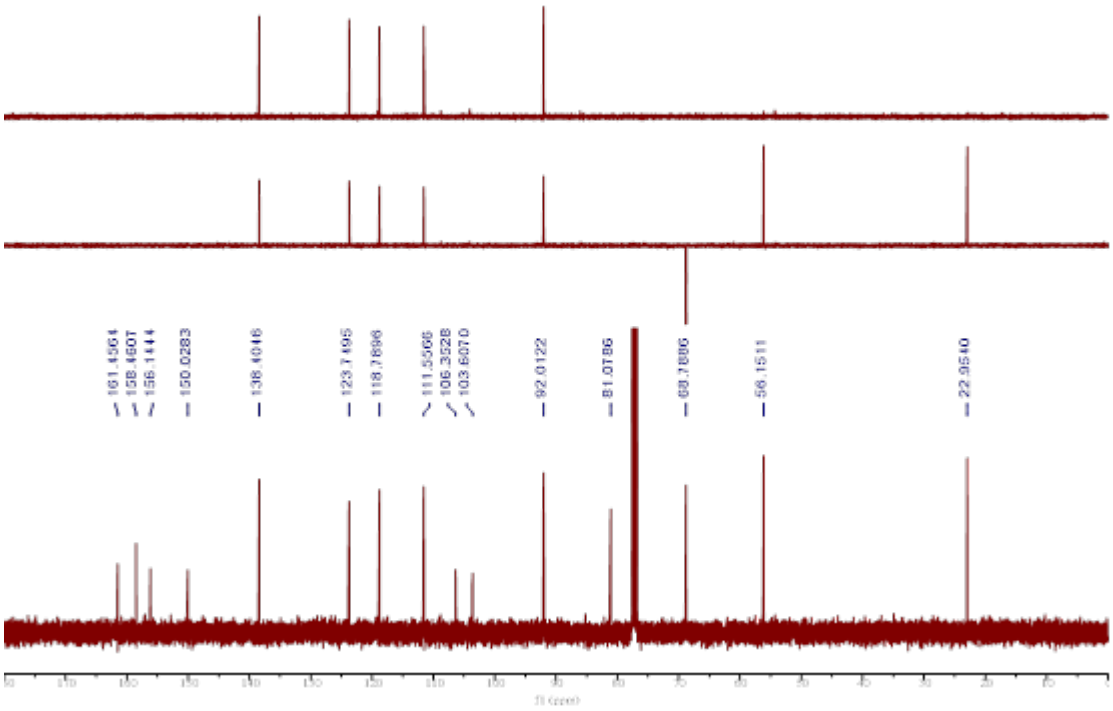

Figure S3. ^{13}C and DEPT NMR of **1**

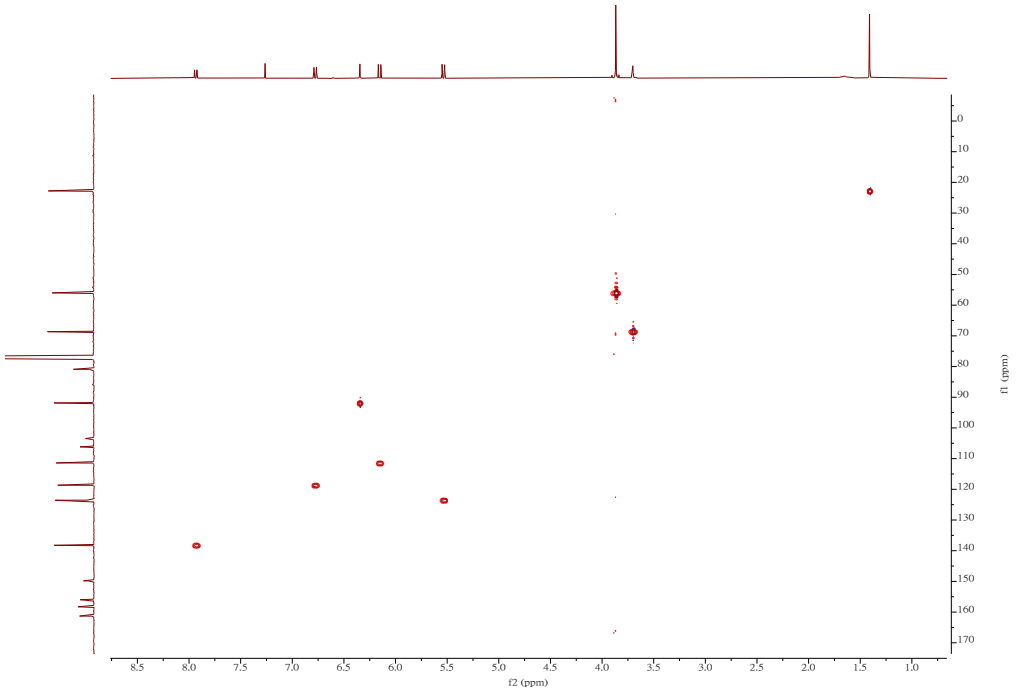

Figure S4. HSQC NMR of **1**

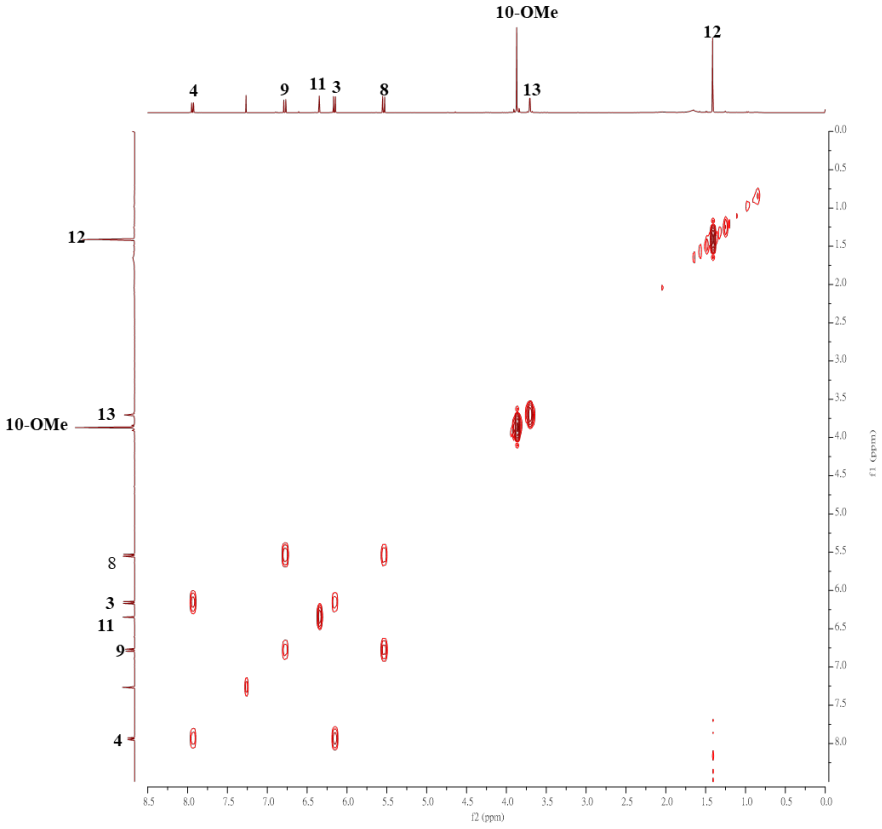

Figure S5. COSY NMR of **1**

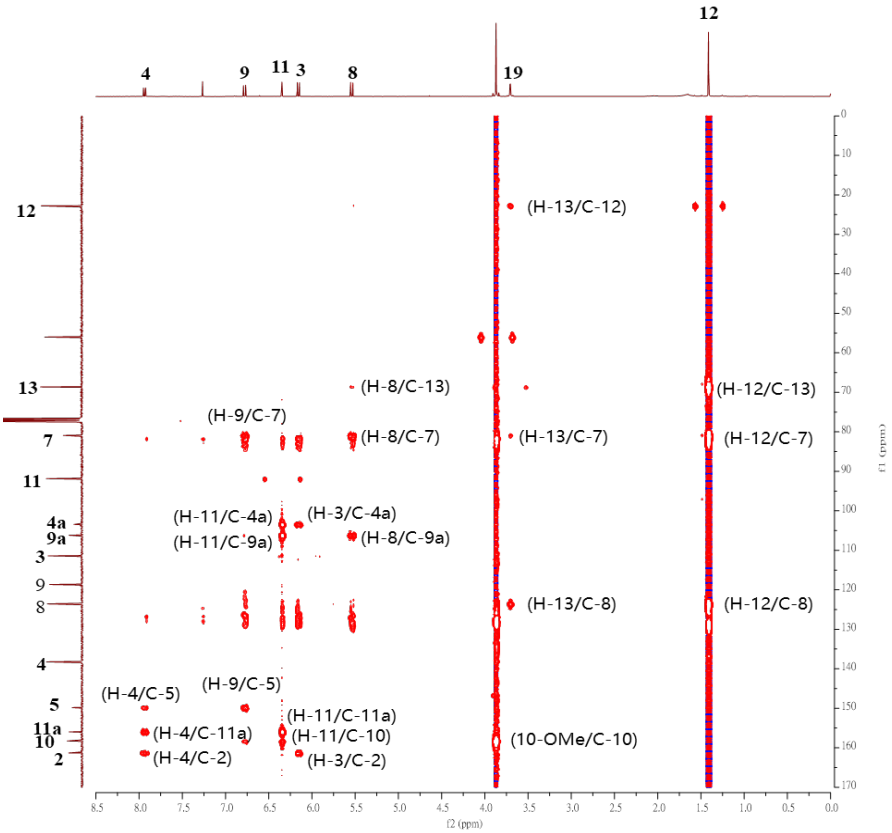

Figure S6. HMBC NMR of **1**

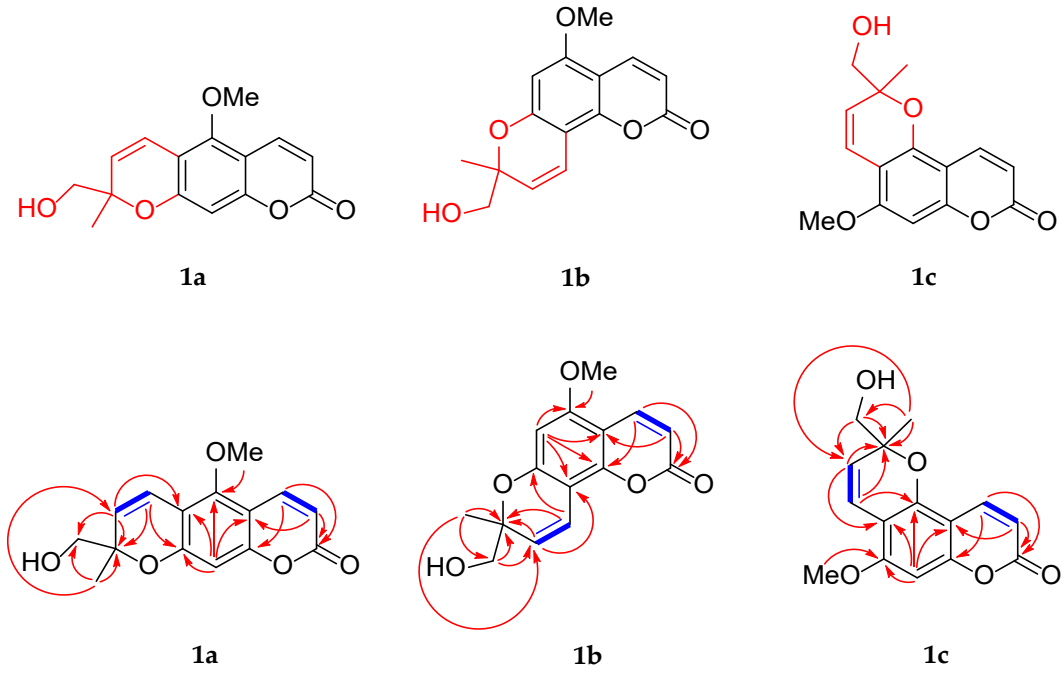

Figure S7. Structures, COSY (bold bond), and HMBC (arrow) correlations of **1a**, **1b**, and **1c**.

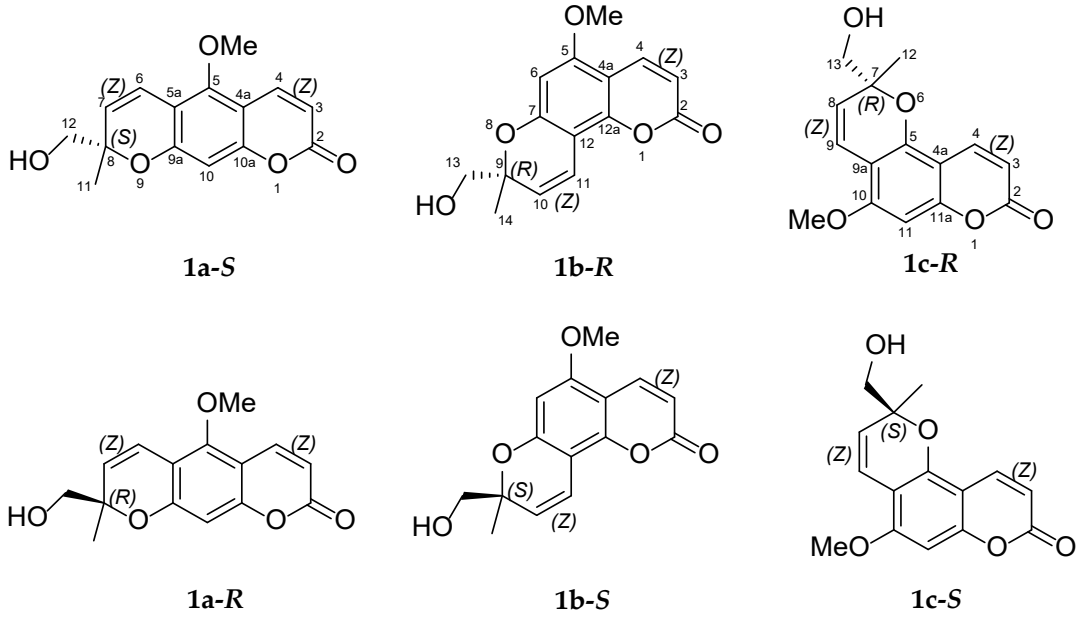

Figure S8. The possible absolute configurations of **1**.

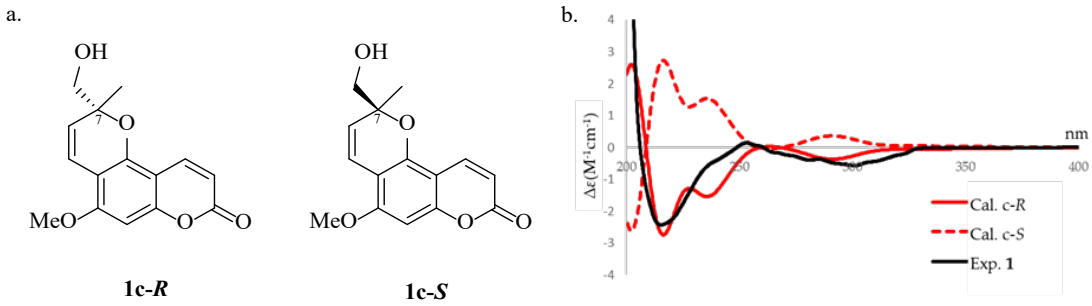

Figure S9. Chemical structures of two **1** isomers and their ECD spectrum.