

## Supporting Information for

Biliatresone, A Reactive Natural Toxin from *Dysphania glomulifera* and *D.*

*littoralis*: Discovery of the Toxic Moiety 1,2-Diaryl-2-Propenone

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## Contents

Abbreviations.....	S-3
General Experimental Procedure .....	S-4
Plant Materials .....	S-4
Extraction, Fractionation, and Isolation .....	S-5
Chemical Structure Elucidations.....	S-6
Biliatresone ( <b>1</b> ) .....	S-6
Betavulgarin ( <b>2</b> ).....	S-7
(3 <i>S</i> ) 2'-Hydroxy-5-methoxy-6,7-methylenedioxyisoflavanone ( <b>3</b> ) .....	S-8
Humeone, 1,2-Methylenedioxy-4-methoxy-seco-pterocarpan ( <b>4</b> ) .....	S-8
3'-Methoxy-biliatresone ( <b>1m</b> ).....	S-9
Demethylene biliatresone ( <b>1d</b> ) .....	S-9
Synthesis of 1,2-diaryl-2-propen-1-one ( <b>5</b> ) .....	S-10
Zebrafish Toxicity Assay .....	S-10
References.....	S-11

## Supplementary Data

Figure S1. Pictures of toxic plant and the pasture.....	S-12
Figure S2. Isolation scheme of compounds ( <b>1-4</b> ) and yield of toxic components .....	S-13
Figure S3. <sup>1</sup> H NMR and <sup>1</sup> H- <sup>1</sup> H cosy spectroscopic data for <b>1</b> .....	S-14
Figure S4. <sup>13</sup> C NMR and DEPT135 spectroscopic data for <b>1</b> .....	S-15
Figure S5. HMQC spectrum data for <b>1</b> .....	S-16
Figure S6. HMBC spectrum data for <b>1</b> .....	S-17
Figure S7. NOESY spectrum data for <b>1</b> .....	S-18
Figure S8. HRMS and FT-IR spectroscopic data for <b>1</b> .....	S-19
Figure S9. RP-HPLC and LC-MS analysis for <b>2</b> .....	S-20
Figure S10. <sup>1</sup> H NMR and HRMS spectroscopic data for <b>2</b> .....	S-21
Figure S11. RP-HPLC and LC-MS analysis for <b>3</b> .....	S-22
Figure S12. <sup>1</sup> H NMR and <sup>1</sup> H- <sup>1</sup> H cosy spectroscopic data for <b>3</b> .....	S-23
Figure S13. <sup>13</sup> C NMR and DEPT135 spectroscopic data for <b>3</b> .....	S-24
Figure S14. HMQC spectrum data for <b>3</b> .....	S-25
Figure S15. HMBC spectrum data for <b>3</b> .....	S-26
Figure S16. HRMS and FT-IR spectroscopic data for <b>3</b> .....	S-27

Figure S17.	CD spectrum for <b>3</b> .....	S-28
Figure S18.	RP-HPLC and LC-MS analysis for <b>4</b> .....	S-29
Figure S19.	<sup>1</sup> H NMR and <sup>13</sup> C NMR spectroscopic data for <b>4</b> .....	S-30
Figure S20.	HMQC spectrum data for <b>4</b> .....	S-31
Figure S21.	HMBC spectrum data for <b>4</b> .....	S-32
Figure S22.	HRMS and FT-IR spectroscopic data for <b>4</b> .....	S-33
Figure S23.	LC-MS analysis for <b>1w</b> , <b>1m</b> , <b>1d</b> , and <b>1</b> .....	S-34
Figure S24.	RP-HPLC analysis for <b>1w</b> , <b>1m</b> , <b>1d</b> , and <b>1</b> in MeOH- and EtOH-based solvents .....	S-35
Figure S25.	<sup>1</sup> H NMR spectrum for a mixture of <b>1m</b> and <b>1</b> .....	S-36
Figure S26.	<sup>13</sup> C NMR spectrum data for a mixture of <b>1m</b> and <b>1</b> .....	S-37
Figure S27.	DEPT135 spectrum data for a mixture of <b>1m</b> and <b>1</b> .....	S-38
Figure S28.	HMQC spectrum data for a mixture of <b>1m</b> and <b>1</b> .....	S-39
Figure S29.	HMBC spectrum data for a mixture of <b>1m</b> and <b>1</b> .....	S-40
Figure S30.	HRMS spectroscopic data of <b>1m</b> and <b>1</b> .....	S-41
Figure S31.	LC-MS analysis of the minor peak <b>1d</b> with <b>1m</b> and <b>1</b> for collection.....	S-42
Figure S32.	<sup>1</sup> H NMR and HMBC spectroscopic data for the purified <b>1d</b> .....	S-43
Figure S33.	<sup>1</sup> H NMR, <sup>13</sup> C NMR, and DEPT 135 spectroscopic data for the synthetic <b>5</b> .....	S-44
Figure S34.	LC-MS analysis for the synthetic <b>5</b> and its MeOH adduct.....	S-45
Table S1.	1D and 2D NMR data of compounds <b>1m</b> and <b>1</b> .....	S-46
Table S2.	1D and 2D NMR data of compounds <b>3</b> and <b>4</b> .....	S-47

**Abbreviations:**

ACN	Acetonitrile
ASAP	Atmospheric Solids Analysis Probe
CD	Circular Dichroism
COSY	Correlation Spectroscopy
DAD	Diode Array Detector
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
dpf	Days Post-Fertilization
ESI	Electro Spray Ionization
EtOAc	Ethyl Acetate
EtOH	Ethanol
FT-IR	Fourier Transform Infrared
Fr	Fraction
Hex	Hexane
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
LC-MS	Liquid Chromatography-Mass Spectrometry
MeOH	Methanol
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
ODS	Octadecyl-Silica
Pet	Petroleum
RP-HPLC	Reverse Phase-High Performance Liquid Chromatography
Subfr	Sub-fraction
t <sub>R</sub>	Retention Time
UV	Ultraviolet

## General Experimental Procedures

HPLC-grade solvents (petroleum ether, hexane, CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, EtOH, MeOH, ACN, and water) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used in all processes, including RP-HPLC and LC-MS. Synthesis reagents (1,2-diaryl-ethanone, piperidine, acetic acid, formalin (37%), and NaHCO<sub>3</sub>) were purchased from Sigma-Aldrich (St. Louis, MO, USA). RP-HPLC was performed at room temperature in an Agilent 1100 series system (Santa Clara, CA, USA) equipped with vacuum degasser, quaternary pump, thermostatically-controlled column compartment, and DAD. LC-MS (ESI, positive mode, *m/z*) spectra were collected on a Shimadzu LCMS-2010EV system (Kyoto, Japan) with UV detector.

The chemical structures were elucidated by the analytical spectroscopic techniques of FT-IR, CD, HRMS, <sup>1</sup>H NMR, <sup>13</sup>C NMR, DEPT135, and 2D NMR techniques (<sup>1</sup>H-<sup>1</sup>H COSY, HMQC, HSQC, NOESY, and HMBC). FT-IR was recorded as a thin film on a ZnSe plate with a Nicolet Avatar 370DTGS spectrometer (Thermo Electron Corporation). CD spectra were measured at 25°C and a speed of 1 nm·s<sup>-1</sup> as a thin film in a demountable rectangular cell with path length of 0.5 mm in an AVIV model 410 spectrometer (AVIV Biomedical, Inc. Lakewood, NJ, USA) equipped with a temperature controller. To obtain HRMS, ASAP (ESI, positive mode) was operated in a Thermo Scientific Exactive spectrometer powered by Orbitrap technology. The differences (*ppm*) value between a found (*F*) and a calculated value (*C*) were calculated from the equation  $ppm \text{ Difference} = |F-C|/C \times 10^6$ . The NMR spectra were acquired on a Bruker AVANCE 400 or 400II spectrometer with <sup>1</sup>H and <sup>13</sup>C frequencies of 400 and 100 MHz, respectively. Chemical shifts were in *ppm* and relative to tetramethylsilane; coupling constants (*J*) were reported in *Hz*. The NMR lock solvents (acetone-d<sub>6</sub> and CD<sub>3</sub>OD) were purchased from Cambridge Isotope Laboratories.

## Plant Materials

A sample (~2.8 kg) containing a mixture of *D. glomulifera* and *D. littoralis* were collected from the area

of the Murray river, along the Hume Weir, near Albury, NSW, Australia (latitude -36.003°, longitude 147.139°) by S.J. Whittaker; the plants were collected from the flats exposed by the drop of the reservoir level during extreme drought. The plants were identified by Surrey Jacobs (Royal Botanic Gardens, Melbourne, Australia). Voucher specimens for the population are housed at the National Herbarium of Victoria (MEL). The plants were frozen until shipment (-18°C), and shipped on dry ice to the authors' laboratories in April 2009 under a plant import permit from Animal Plant Health and Inspection Service, US Department of Agriculture (permit no. P37-08-00668, 21 May 2008). Individual plants were carefully teased from the frozen biomass and preserved as voucher specimens deposited in the herbarium of the University of the Sciences in Philadelphia (PHIL).

### **Extraction, Fractionation, and Isolation**

Freeze-dried plants (100 g) were ground to pass a 20 mesh screen and extracted at room temperature in a blender with 1:1 (v/v) CH<sub>2</sub>Cl<sub>2</sub>/MeOH. As shown in figure S2A, the plant material residue was removed by filtration and extracted twice more in the same solvent to give the CH<sub>2</sub>Cl<sub>2</sub>/MeOH extract. The plant material residue was extracted with water (×3), and the aqueous extracts were pooled as a water extract. After partial evaporation *in vacuo* to about half of the original volume, water was added to the CH<sub>2</sub>Cl<sub>2</sub>/MeOH organic extract and then partitioned with petroleum ether (bp 40-60°C); the pet. ether fractions were pooled. All fractions were evaporated to dryness *in vacuo* (organic) or by lyophilization (aqueous) and subjected to bioassay.

The CH<sub>2</sub>Cl<sub>2</sub>/MeOH extract (Fr1) was subjected to diol silica gel flash column chromatography with hexane, CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, MeOH, and water to generate five solvent fractions partitioned by polarity. The active CH<sub>2</sub>Cl<sub>2</sub> fraction was further separated to seven subfractions through RP-HPLC on a semi-preparative Phenomenex ultracarb 10 ODS-20 (600×10 mm, 10 µm, 5 mL·min<sup>-1</sup>) column in a linear gradient elution of water to MeOH (5 → 95 % MeOH) with DAD detection at 206 and 254 nm. The seven subfractions were taken to dryness *in vacuo* and by lyophilization and submitted to bioassay. The two active subfractions (subFr45) were further purified by semi-preparative RP-HPLC on a YMC

J'sphere ODS-H80 (250×10 mm, 4  $\mu$ m, 3 mL·min<sup>-1</sup>) column in an isocratic solvent system of water/MeOH/ACN (4:3:3, v/v/v) and yielded four compounds (**1-4**). The toxic fractions, subfractions, and individual compounds were analyzed in the RP-HPLC and LC-MS (ESI, positive mode, *m/z*) with a Waters Symmetry C18 column (250×4.6 mm, 5  $\mu$ m, 0.2, 0.3 or 0.5 mL·min<sup>-1</sup>) in the mobile phase of water/MeOH/ACN (4:3:3, v/v/v), water/EtOH/ACN (4:3:3, v/v/v) or water/ACN (1:1, v/v) with UV detection. The toxicity of the six compounds (**1-4**) on the zebrafish larvae was assayed; we confirmed that **1** caused significant biliary dysfunction. The dry weight of the plant material was 6.08% of the frozen weight of the plant; other yield data can be found in figure S2B. The yield of toxic compound **1** was collectively approximately 1.84% of the dry weight of the plant.

Solvent adducts (**1w** and **1m**) and biliatresone (**1**) were purified in the EtOH-based solvent to remove the interference of MeOH and dried directly *in vacuo*. RP-HPLC and LC-MS analysis of the purified compounds were performed with a Waters Symmetry C18 column (250×4.6 mm, 5  $\mu$ m, 0.3 mL·min<sup>-1</sup>) in a mixture of water/EtOH/ACN (4:3:3, v/v/v). For compound **1d**, the tiny peak at *t<sub>R</sub>* 22.18 min or *t<sub>R</sub>* 22.69 was purified by RP-HPLC with a Waters Symmetry C18 column (250×4.6 mm, 5  $\mu$ m, 0.5 mL·min<sup>-1</sup>) in a mixture of water/ACN (1:1, v/v), which leads to higher difference of retention time between **1** and **1d**. This collection was evaporated directly to dryness *in vacuo*. <sup>1</sup>H NMR and HMBC data were acquired in acetone-d<sub>6</sub> lock solvent.

## Chemical Structure Elucidations

Compound **1** was isolated as a yellowish gum. The <sup>13</sup>C NMR spectrum (Figure S4 and Table S1) showed 18 carbons including a carbonyl group ( $\delta_C$ =195.7 ppm, C1'). The DEPT135 spectrum showed two peaks corresponding to dioxymethylene ( $\delta_C$ =102.5 ppm, C1a) and methylene ( $\delta_C$ =130.1 ppm, C3') carbons. Among the 16 protons in the <sup>1</sup>H NMR spectrum, a broad proton peak ( $\delta_H$ =8.05 ppm, OH) was not correlated to a carbon in the HMQC spectrum (Figure S5). This proton showed a correlation to the phenyl carbons ( $\delta_C$ =116.9 ppm, C3'' and  $\delta_C$ =125.9 ppm, C1'') in the HMBC spectrum (Figure S6). An olefinic protons ( $\delta_H$ =6.04, 6.08 ppm, 3'-H) was typical of a  $\alpha$ -methylene with no splitting; these were

correlated with the carbonyl carbon ( $\delta_C=195.7$  ppm, C1') and a quaternary aromatic carbon ( $\delta_C=125.9$  ppm, C1'') in the HMBC. The HMBC spectrum showed correlations from 1a-H to C1 and C2; 3-H to C1, C2, C4 and C5; 3'-H to C1', C2' and C1''; 3''-H to C2'' and C5''; 4''-H to C2'' and C6''; 5''-H to C1'' and C3''; 6''-H to C2', C2'' and C4'' (Figure S6). The NOESY spectrum presented only a cross-peak correlation between 3-H and 4-methoxyl proton (-OCH<sub>3</sub>) (Figure S7).

Biliatresone (**1**): yellowish gum; CD (deg, film):  $\Delta\epsilon = -0.3$  (240 nm),  $\Delta\epsilon = +0.5$  (290 nm); <sup>1</sup>H NMR and <sup>13</sup>C NMR, see Table 1; IR (film):  $\lambda_{\max}$  3379, 2925, 1622, 1485, 1202, 1074, 932, 751 cm<sup>-1</sup>; HPLC-UV/vis (aq MeOH and ACN):  $\lambda_{\max}$  235, 281 nm; HR-ASAP-MS (ESI, positive mode,  $m/z$ ): 329.1022 [M+H]<sup>+</sup> calcd for C<sub>18</sub>H<sub>17</sub>O<sub>6</sub>, 329.1025. Values of <sup>1</sup>H NMR spectroscopy of biliatresone (**1**) measured in the CD<sub>3</sub>OD lock solvent were acquired from biliatresone newly purified in an EtOH-based solvent to compare with values of the <sup>1</sup>H NMR of a mixture of **1** and **1m** in the CD<sub>3</sub>OD lock solvent. <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD, *ppm*):  $\delta_H$  7.13 (m, 2H), 6.81 (m, 2H), 6.39 (s, 1H), 6.07 (s, 1H), 6.01 (s, 1H), 5.94 (s, 2H), 3.95 (s, 3H), 3.70 (s, 3H).

Compound **2** was isolated as a white powder. The HRMS showed a molecular mass of  $m/z$  313.0708 [M+H]<sup>+</sup> (calculated for C<sub>17</sub>H<sub>13</sub>O<sub>6</sub>, 313.0712), indicating a molecular formula of C<sub>17</sub>H<sub>12</sub>O<sub>6</sub>. By comparing our NMR data with the literature (Figures S9-S10), **2** was identified as betavulgarin, 2'-hydroxy-6,7-methylenedioxy-5-methoxyisoflavone.<sup>1</sup>

Betavulgarin (**2**): White powder; <sup>1</sup>H NMR (400 MHz, acetone-d<sub>6</sub>, *ppm*):  $\delta_H$  4.01 (s, 3H), 6.24 (s, 2H), 6.93 (s, 1H), 6.96 (m, 2H), 7.29 (m, 2H), 8.23 (s, 1H), 9.04 (s, OH); HPLC-UV/vis (aq MeOH and ACN):  $\lambda_{\max}$  218, 246, 324 nm; LC-MS (ESI, positive mode,  $m/z$ ): 313 [M+H]<sup>+</sup>, 376 [M+Cu]<sup>+</sup>; HR-ASAP-MS (ESI, positive mode,  $m/z$ ): 313.0708 [M+H]<sup>+</sup> calcd for C<sub>17</sub>H<sub>13</sub>O<sub>6</sub>, 313.0712.



Compound **3** was isolated as a yellowish gum. LC-MS analysis gave a mass of  $m/z$  315  $[M+H]^+$  indicating a molecular formula of  $C_{17}H_{14}O_6$ ; this was confirmed by the HR-MS analysis ( $m/z$  315.0864  $[M+H]^+$ ; calculated for  $C_{17}H_{14}O_6$ , 315.0869) (Figures S11 and S16). UV spectrum and a three proton spin system, 3-H ( $\delta_H=4.11$  ppm), 2 $\alpha$ -H ( $\delta_H=4.51$  ppm), and 2 $\beta$ -H ( $\delta_H=4.66$  ppm), of the  $^1H$  NMR data were typical of an isoflavanone skeleton (Figures S11 and S12). Seventeen carbons in the  $^{13}C$  NMR and DEPT135 spectra, including the dioxymethylene carbon ( $\delta_C=102.9$  ppm, C6a), the methoxyl carbon ( $\delta_C=60.8$  ppm) and the methylene carbon ( $\delta_C=71.0$  ppm, C2), were also typical of an isoflavanonoid (Figure S13). The hydroxy proton ( $\delta_H=8.73$  ppm, OH) showed correlations with the C1' and C2' carbons of the B ring in the HMBC spectrum (Figure S15). From 1D and 2D analysis (Table S2) along with the CD analysis suggested that **3** has the *S*-stereoisomer configuration, with a negative Cotton effect between 290 and 340 nm, corresponding to a  $n\rightarrow\pi^*$  transition energy (Figure S17), we identified the chemical structure of **3** as (3*S*) 2'-hydroxy-5-methoxy-6,7-methylenedioxy isoflavanone, not previously reported.

(3*S*) 2'-Hydroxy-5-methoxy-6,7-methylenedioxyisoflavanone (**3**): Yellowish gum; CD (deg, film):  $\Delta\epsilon = 1.5$  (180 nm),  $\Delta\epsilon = -1.2$  (244 nm),  $\Delta\epsilon = -0.27$  (310 nm);  $^1H$  NMR and  $^{13}C$  NMR, see Table 2; IR (film):  $\nu_{max}$  3575, 2918, 2850, 1625, 1475, 1250, 1100, 930, 776  $cm^{-1}$ ; HPLC-UV/vis (aq MeOH and ACN):  $\lambda_{max}$  245, 283, 342 nm; LC-MS (ESI, positive mode,  $m/z$ ): 315  $[M+H]^+$ , 378  $[M+Cu]^+$ ; HR-ASAP-MS (ESI, positive mode,  $m/z$ ): 315.0864  $[M+H]^+$  calcd for  $C_{17}H_{14}O_6$ , 315.0869.

Compound **4** was isolated as a yellowish gum and purified by RP-HPLC (Table S2). The LC-MS analysis showed a major peak at  $m/z$  329  $[M+H]^+$ , leading to a molecular formula of  $C_{18}H_{16}O_6$ , verified by the mass at  $m/z$  328.0945  $[M]^+$  (calculated for  $C_{18}H_{16}O_6$ , 328.0947) in the HR-MS data (Figures S18 and S22). The  $^1H$  NMR spectrum displayed resonances for two aromatic ring protons in a di-substituted phenyl moiety ( $\delta_H=7.2$ -7.8 ppm, 4'-H, 5'-H, 6'-H, 7'-H) and a phenyl moiety with the dioxymethylene group at ( $\delta_H=6.03$  ppm, 1a-H), together with a methine proton ( $\delta_H=6.54$  ppm, 3-H) and two methoxyl protons

( $\delta_{\text{H}}$ =3.72, 3.89 ppm) (Figure S19). The  $^{13}\text{C}$  NMR and HMQC spectra exhibited resonances for 18 carbons, including the dioxymethylene carbon ( $\delta_{\text{C}}$ =102.5 ppm, C1a), two methoxyl carbons ( $\delta_{\text{C}}$ =60.4, 57.0 ppm), a methine carbon ( $\delta_{\text{C}}$ =90.1 ppm, C3), and a primary alcohol ( $\delta_{\text{C}}$ =56.4 ppm) (Figures S19 and S20). In the HMBC spectrum, the methine proton ( $\delta_{\text{H}}$ =6.54 ppm, 3-H) showed correlations to the dioxymethylene carbons, C1 and C2 ( $\delta_{\text{C}}$ =131.6, 152.2 ppm), C4 ( $\delta_{\text{C}}$ =155.9 ppm), and C5 ( $\delta_{\text{C}}$ =104.9 ppm) (Figure S21). The primary alcohol proton ( $\delta_{\text{H}}$ =4.58 ppm) showed correlations with three carbons, C3' ( $\delta_{\text{C}}$ =119.9 ppm), C2' ( $\delta_{\text{C}}$ =147.5 ppm) and C3'a ( $\delta_{\text{C}}$ =130.0 ppm), in the di-substituted phenyl ring. From 1D and 2D analysis (Table S2), the structure of **4** was identified as a novel seco-pterocarpan, 1,2-methylenedioxy-4-methoxy-seco-pterocarpan. We have given **4** the trivial name of 'humeone' in recognition of plant collection along the Hume Weir.

1,2-Methylenedioxy-4-methoxy-seco-pterocarpan (**4**): Yellowish gum; CD (deg, film):  $\Delta\epsilon = -3.6$  (195 nm),  $\Delta\epsilon = +0.2$  (300 nm);  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Table 2; IR (film)  $\nu_{\text{max}}$  2912, 2843, 1468, 1440, 1202, 1074, 932, 739  $\text{cm}^{-1}$ ; HPLC-UV/vis (aq MeOH and ACN):  $\lambda_{\text{max}}$  245, 294 nm; LC-MS (ESI, positive mode,  $m/z$ ): 311  $[\text{M}-\text{H}_2\text{O}]^+$ , 329  $[\text{M}+\text{H}]^+$ ; HR-ASAP-MS (ESI, positive mode,  $m/z$ ): 328.0945  $[\text{M}]^+$  calcd for  $\text{C}_{18}\text{H}_{16}\text{O}_6$ , 328. 0947.

3'-Methoxy-biliatresone (**1m**): yellowish gum;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Table 1; HPLC-UV/vis (aq MeOH and ACN):  $\lambda_{\text{max}}$  281, 310 nm; HRMS (ESI, positive mode,  $m/z$ ): 361.1270  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{19}\text{H}_{20}\text{HO}_7$ , 361.1287; LC-MS (ESI, positive mode,  $m/z$ ): 361  $[\text{M}+\text{H}]^+$  (calcd for  $\text{C}_{19}\text{H}_{21}\text{O}_7$ ), and 383  $[\text{M}+\text{Na}]^+$  (calcd for  $\text{C}_{19}\text{H}_{20}\text{NaO}_7$ ).

Demethylene biliatresone (**1d**):  $^1\text{H}$  NMR (400MHz, acetone- $d_6$ , ppm):  $\delta_{\text{H}}$  7.11 (t,  $J=8$ , 1H), 7.06 (d,  $J=7.6$ , 1H), 6.84 (d,  $J=8$ , 1H), 6.77 (t,  $J=7.6$ , 1H), 6.43 (s, 1H), 5.97 (s, 2H), 4.03 (s, 2H), 3.86 (s, 3H), 3.76 (s, 3H); HPLC-UV/vis (aq MeOH and ACN):  $\lambda_{\text{max}}$  285, 267 nm; LC-MS (ESI, positive mode,  $m/z$ ): 317

$[M+H]^+$  (calculated for  $C_{17}H_{17}O_6$ , 317.103).

### Synthesis of 1,2-diaryl-2-propen-1-one (5)

The 1,2-diaryl-ethanone (5 g, 25.5mmol) in MeOH (45 mL) was treated with piperidine (0.2 mL), acetic acid (0.2 mL) and 37% formalin (5.4 mL). The mixture was refluxed for 3 h at 95-100°C. After cooling, ice-cold 3%  $NaHCO_3$  was gradually added into the reaction mixture to pH 7.4 (approx. 150mL). The reactant was put in the refrigerator for 18 h to precipitate the semi-solid product. Supernatant was removed and then added water and  $CH_2Cl_2$  to liquid-liquid extraction. The  $CH_2Cl_2$  fraction containing the product was collected and dried. It was a colorless oil (94% yield).<sup>2</sup>

1,2-Diaryl-2-propen-1-one (**5**): colorless oil;  $^1H$  NMR (400MHz, acetone- $d_6$ , ppm):  $\delta_H$  7.99 (d,  $J=8.2$ , 2H), 7.52 (dd  $J=7.4$ , 4H), 7.38 (m, 4H), 6.15 (s, 1H), 5.63 (s, 1H);  $^{13}C$  NMR (100 MHz, acetone- $d_6$ , ppm):  $\delta_C$  196.9, 148.3, 137.3, 137.2, 133.2, 129.8, 128.8 128.7, 127.1, 120.4; LC-MS (ESI, positive mode,  $m/z$ ): 209  $[M+H]^+$  (calcd for  $C_{15}H_{13}O$ ) and 231  $[M+Na]^+$  (calcd for  $C_{15}H_{12}NaO$ ).

### Zebrafish Toxicity Assay

Plant extracts, fractions and compounds were suspended in anhydrous DMSO to give stock samples. Zebrafish larvae reared in 24- and 96-well plates were exposed to the sample for 24-72 h (final concentration 1% DMSO in the medium) with various concentrations. For the initial extract and fractions, concentrations of 5-50  $\mu g/ml$  were tested. The final compounds were tested at 0.0625-1.0  $\mu g/ml$ . The embryo medium with extract was exchanged daily. Following treatment of the zebrafish larva, the fluorescent long chain fatty acid Bodipy-C16 (Invitrogen) was added to the water as previously described.<sup>3</sup> Gallbladder and intestinal fluorescence in the treated and control larva were examined with a stereomicroscope (Olympus MVX-10). All assays were performed in triplicate and controls for swallowing were performed with each sample.

## References

- (1) Elliger, C. A. and Halloin, J. M. (1994) Phenolics induced in *Beta vulgaris* by *Rhizoctonia solani* infection. *Phytochemistry* 37, 691–693.
- (2) Tramontini, M. (1973) Advances in the chemistry of Mannich bases. *Synthesis* 12, 703–775.
- (3) Lorent, K., Moore, J. C., Siekmann, A. F., Lawson, N., and Pack, M. (2010) Reiterative use of the notch signal during zebrafish intrahepatic biliary development. *Dev. Dynam.* 239, 855–864.

## Supplementary Data

A.

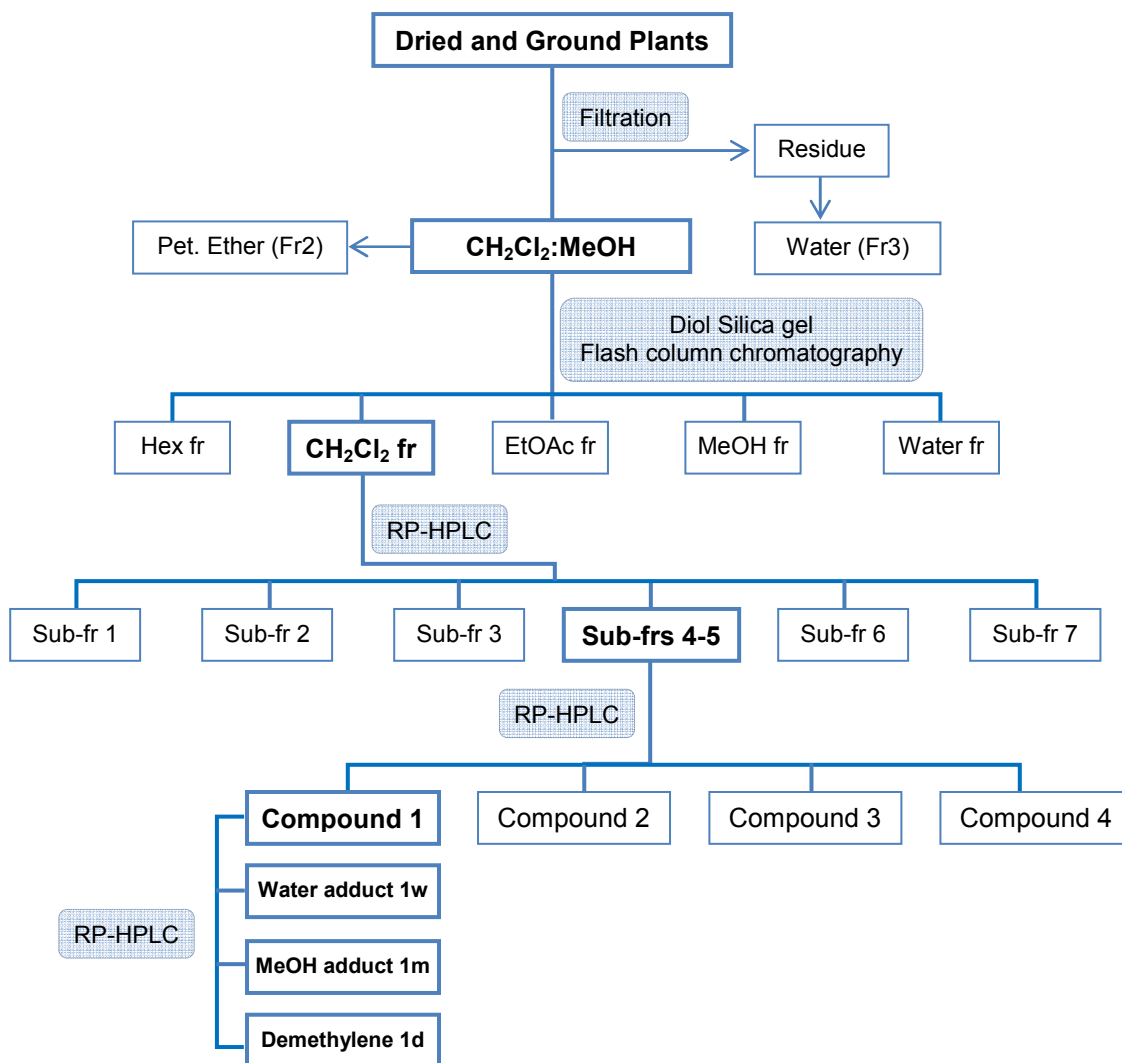


B.



**Figure S1.** The toxic plants *Dysphania glomulifera* and *D. littoralis*. (A) We collected the plants from the pasture associated with the 2007 outbreak. The Murray River reservoir impounded by the Hume Dam (formerly the Hume Weir) is visible in the background. (B) A grouping of the plants at the collection site. *D. glomulifera* and *D. littoralis* often grow together and can be differentiated only microscopically. Images courtesy of author Steven J. Whittaker, 2007.

A.

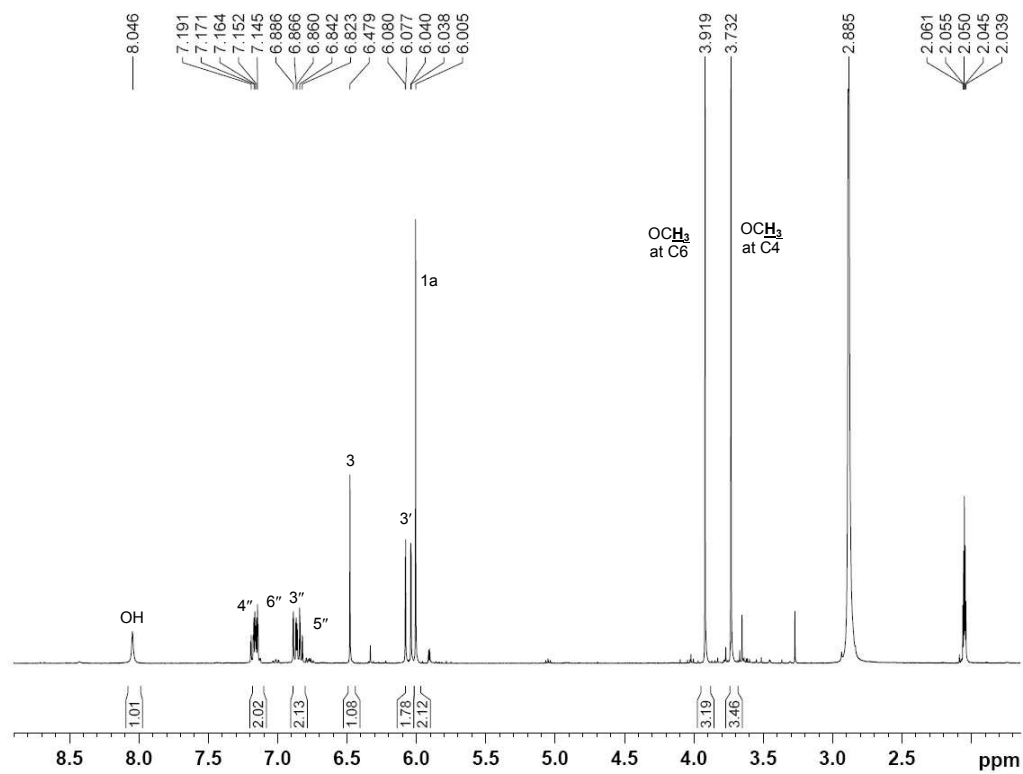


B.

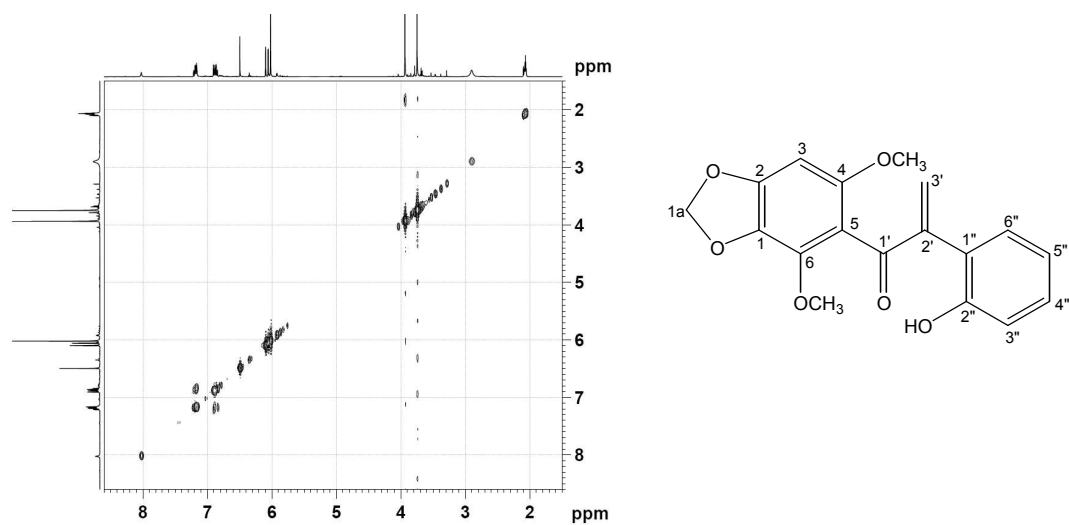
Toxic components	% of dry weight
Dry weight	100.00
CH <sub>2</sub> Cl <sub>2</sub> :MeOH extract	40.05
CH <sub>2</sub> Cl <sub>2</sub> fraction (Fr1)	8.53
Subfractions 4 and 5 (subFr45)	4.08
<b>1 and its derivatives (1w, 1m, and 1d)</b>	1.84

**Figure S2.** The isolation scheme of compounds (**1-4**) (A) and yield of the toxic components (B). The bolded fractions and compounds showed toxicity in the zebrafish assay.

A.

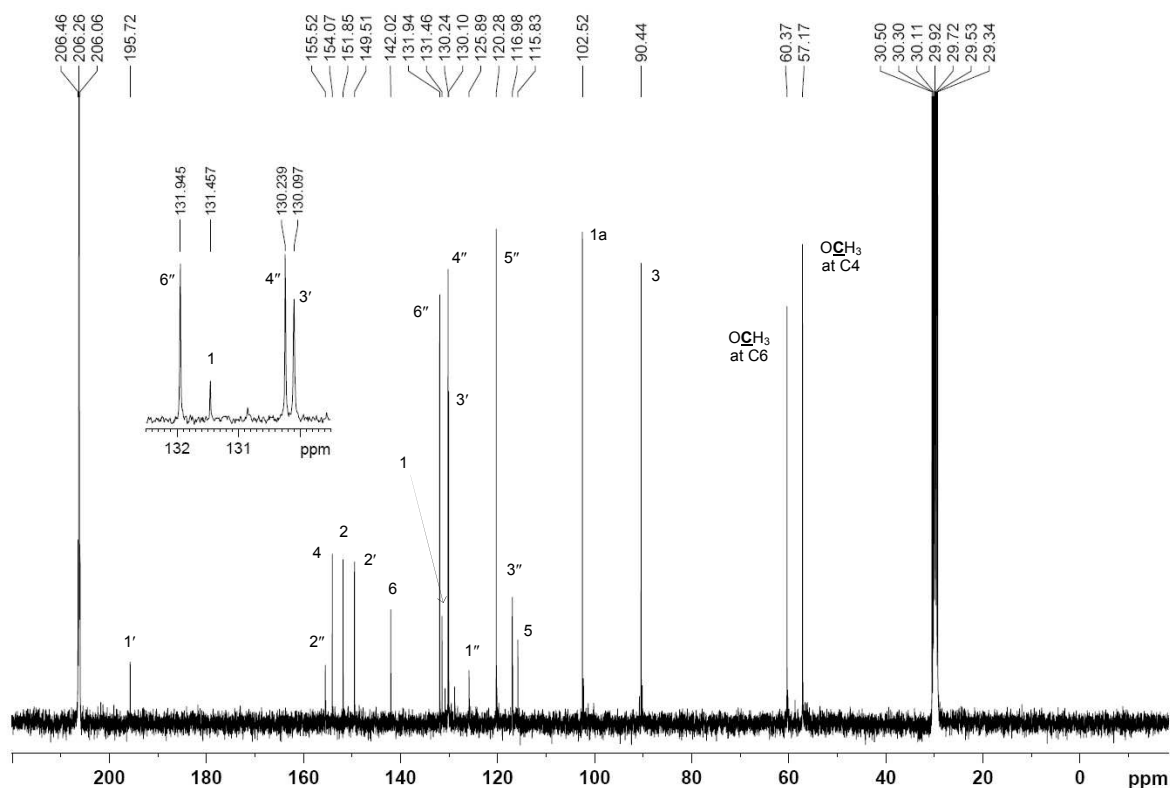


B.

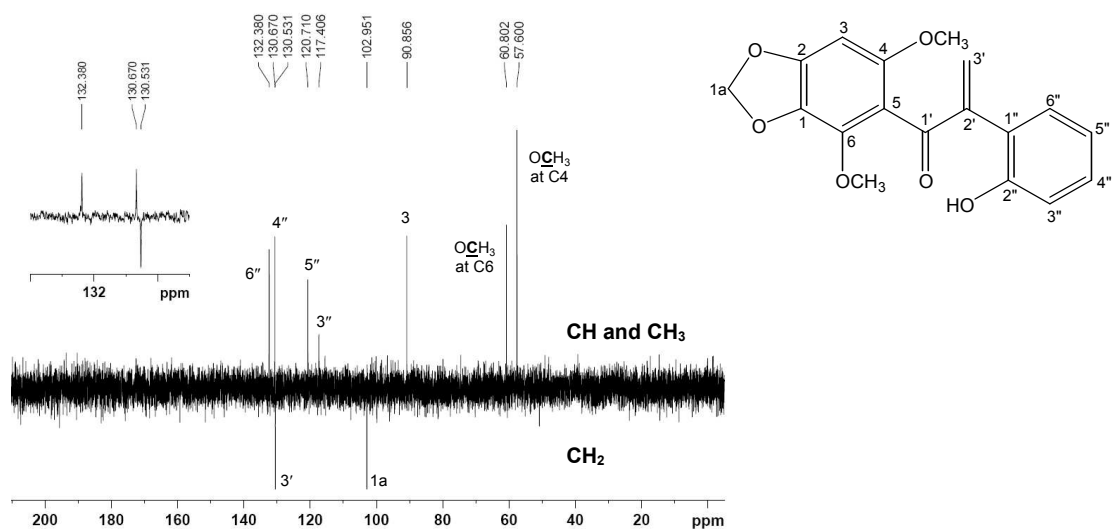


**Figure S3.**  $^1\text{H}$  NMR spectrum (A) and  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (B) for **1** (400 MHz, acetone- $\text{d}_6$ ).

A.

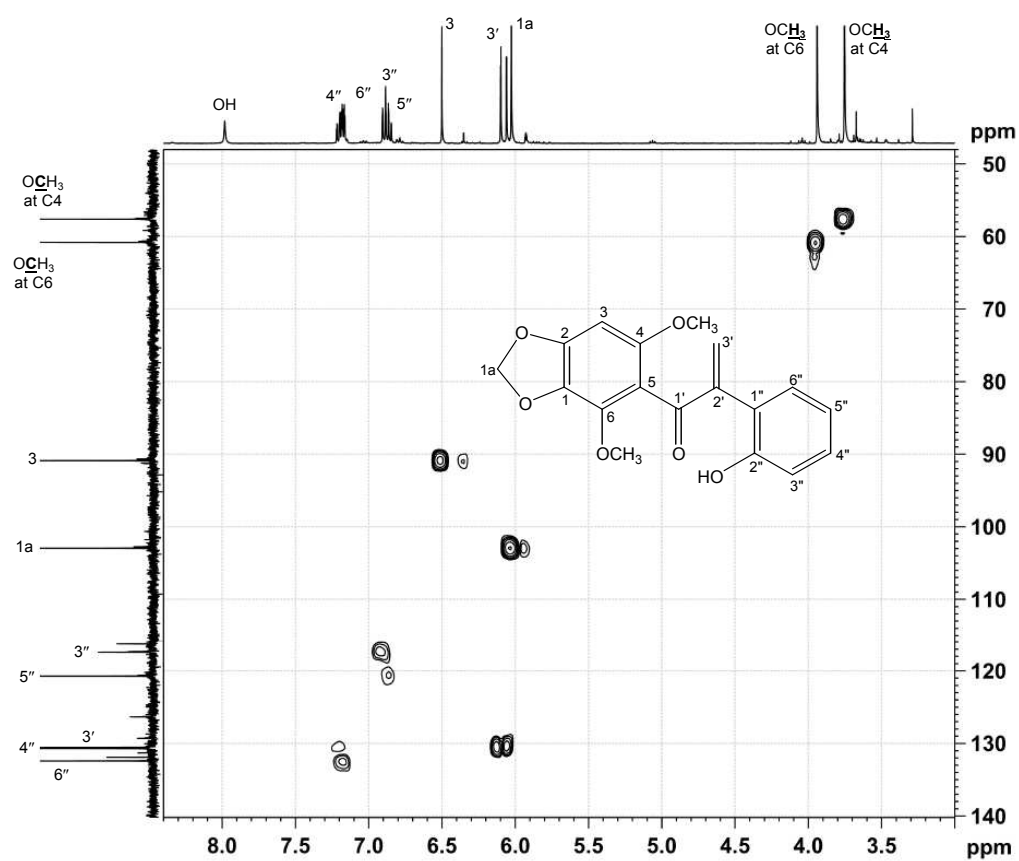


B.

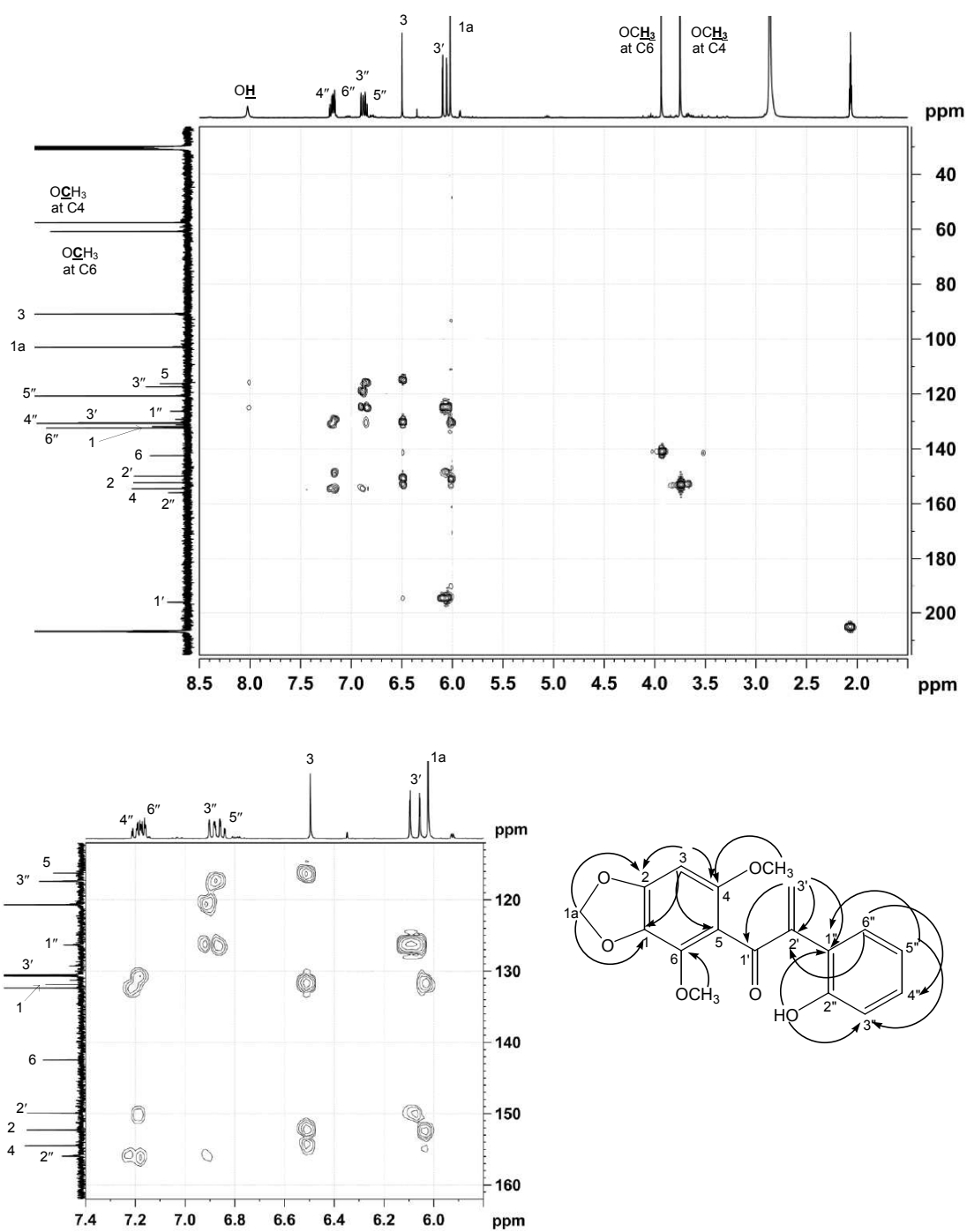


**Figure S4.**  $^{13}\text{C}$  NMR spectrum (A) and DEPT 135 spectrum (B) for **1** (100 MHz, acetone- $\text{d}_6$ ).

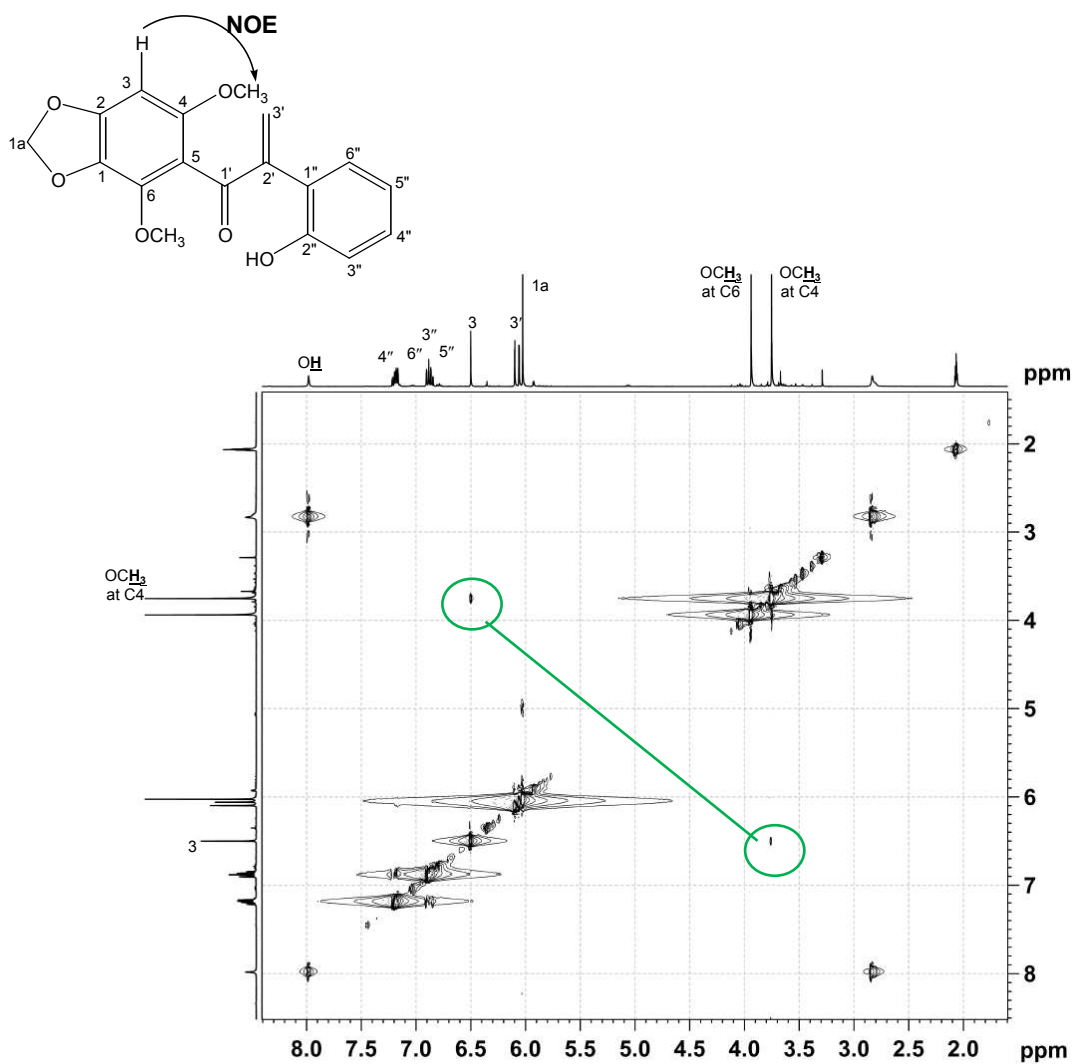




**Figure S5.** HMQC spectrum for **1** (100 MHz, acetone- $\text{d}_6$ ).



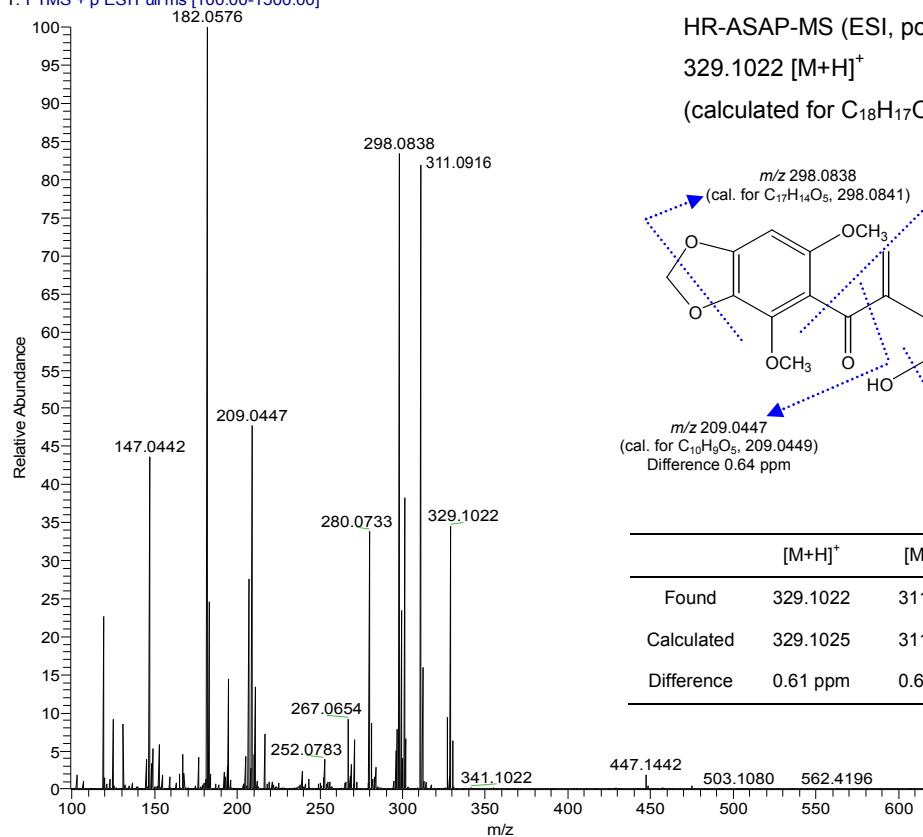
**Figure S6.** HMBC spectrum for **1** (100 MHz, acetone- $d_6$ ). Major interactions are indicated in the compound structure.



**Figure S7.** NOESY spectrum for **1** (400 MHz, acetone- $d_6$ ). The major NOE interaction is shown by the green line connecting the circled resonances (H→H NOE).

A.

jp245-4-200 degree temp #2-17 RT: 0.03-0.23 AV: 16 NL: 1.15E8  
T: FTMS + p ESI Full ms [100.00-1500.00]



B.

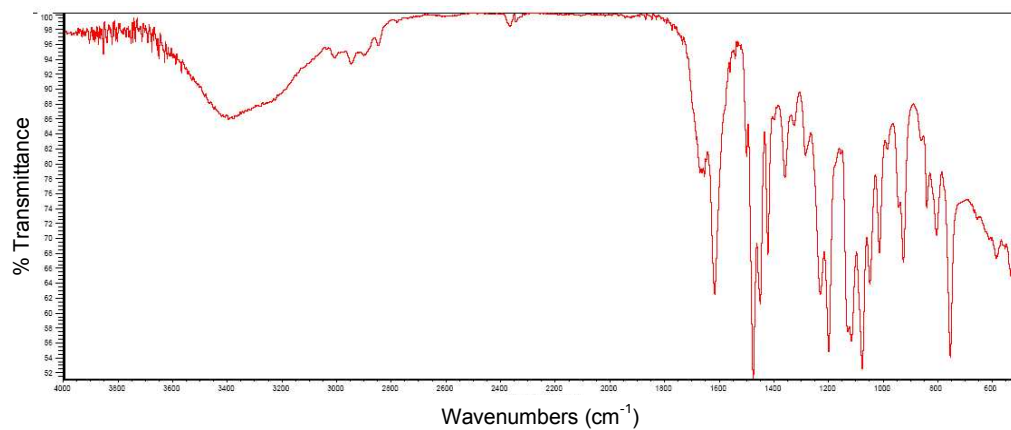
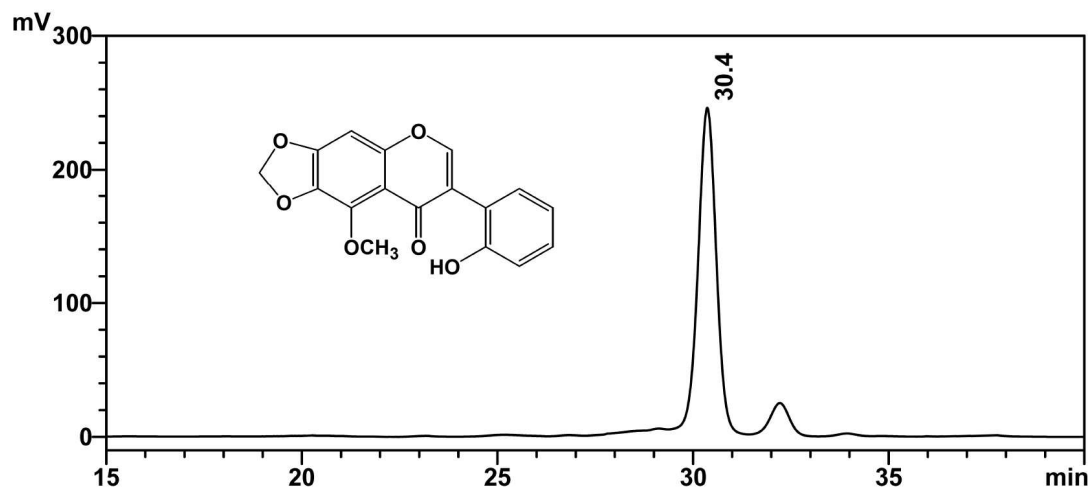


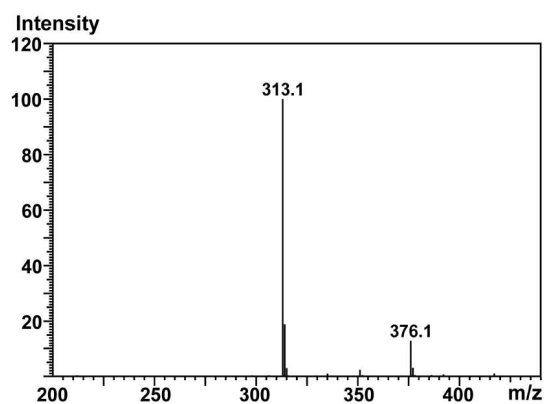
Figure S8. HRMS spectrum (A) and FT-IR spectrum (B) for 1.

A.

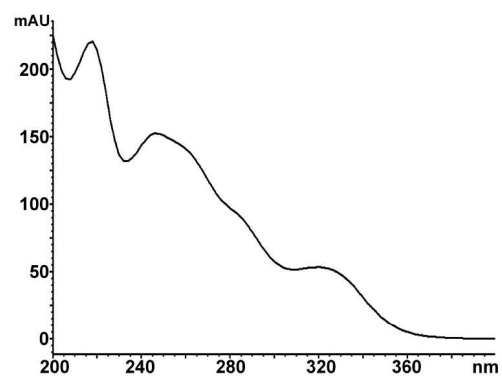


B.

Ret.Time : 30.6 / Base Peak : 313.1 / Polarity : Pos

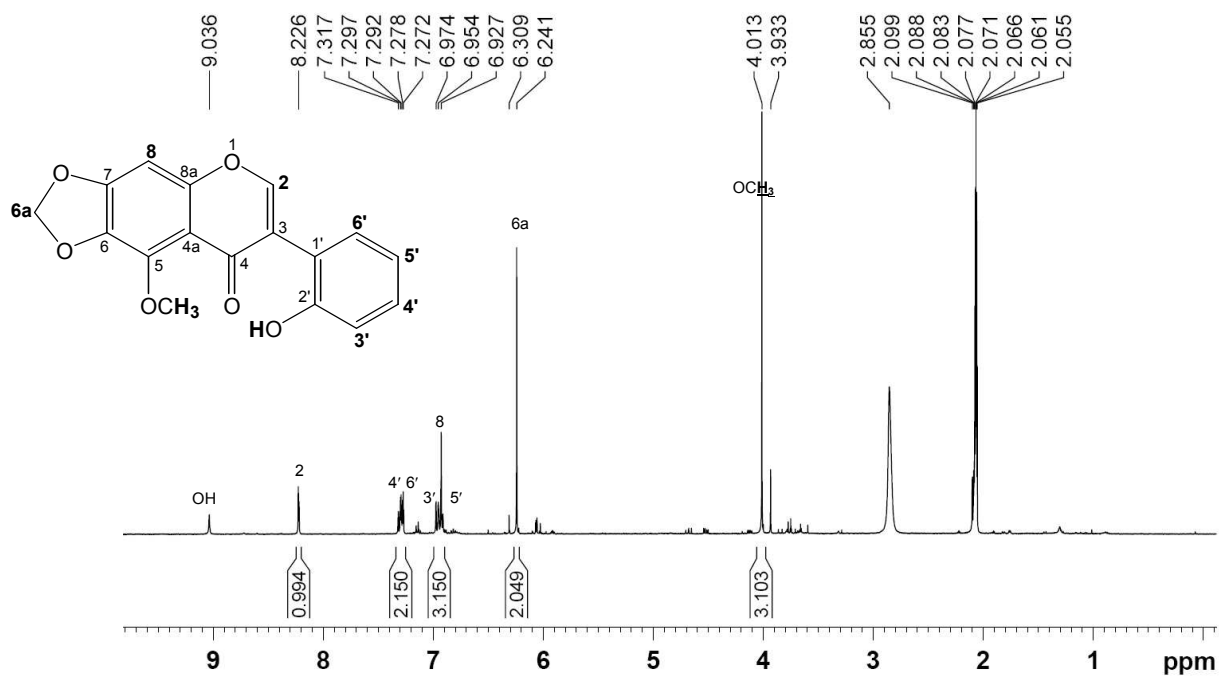


C.



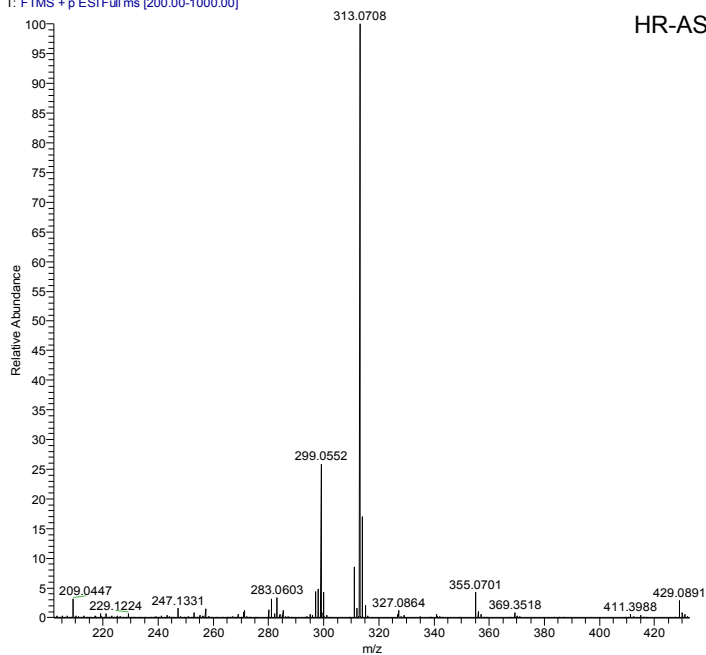
**Figure S9.** RP-HPLC spectrum (A) with DAD detection and LC-MS analysis (B) for **2**. (C) The UV spectrum from the DAD data.

A.

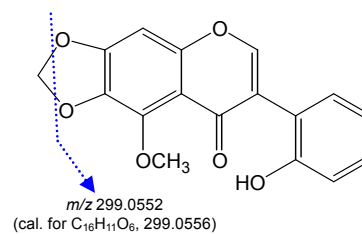


B.

jrp245-2 #17 RT: 0.24 AV: 1 NL: 1.25E8  
T: FTMS + p ESI Full ms [200.00-1000.00]



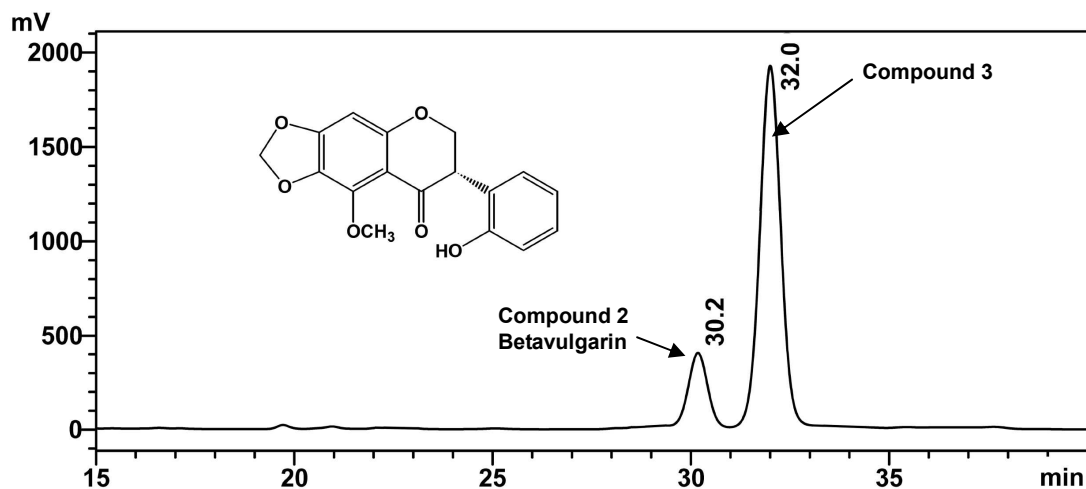
HR-ASAP-MS (ESI, positive ion, *m/z* 313.0708 [M+H]<sup>+</sup>  
(calculated for C<sub>17</sub>H<sub>13</sub>O<sub>6</sub>, 313.0712)



	[M+H] <sup>+</sup>	[M-CH <sub>2</sub> ] <sup>+</sup>
Found	313.0708	299.0552
Calculated	313.0712	299.0556
Difference	1.59 ppm	1.59 ppm

**Figure S10.** <sup>1</sup>H NMR spectrum (400 MHz, acetone-d<sub>6</sub>) (A) and HRMS spectrum (B) for **2**.

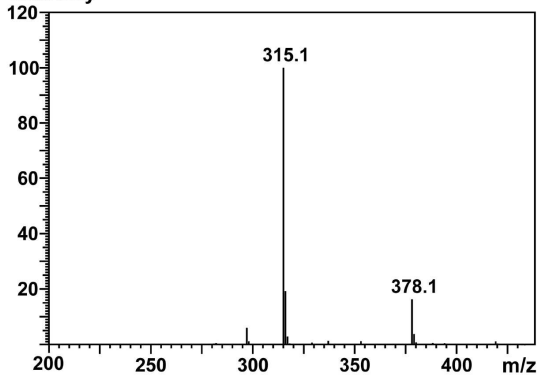
A.



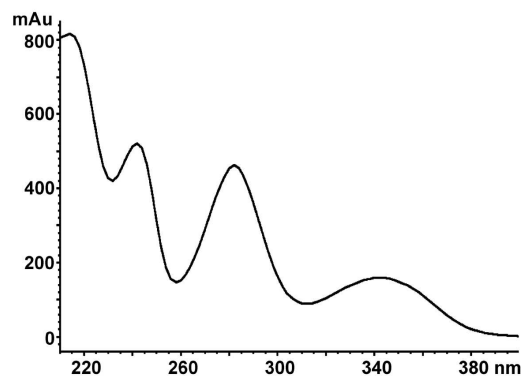
B.

Ret.Time : 32.2 / Base Peak : 315.1 / Polarity : Pos

Intensity

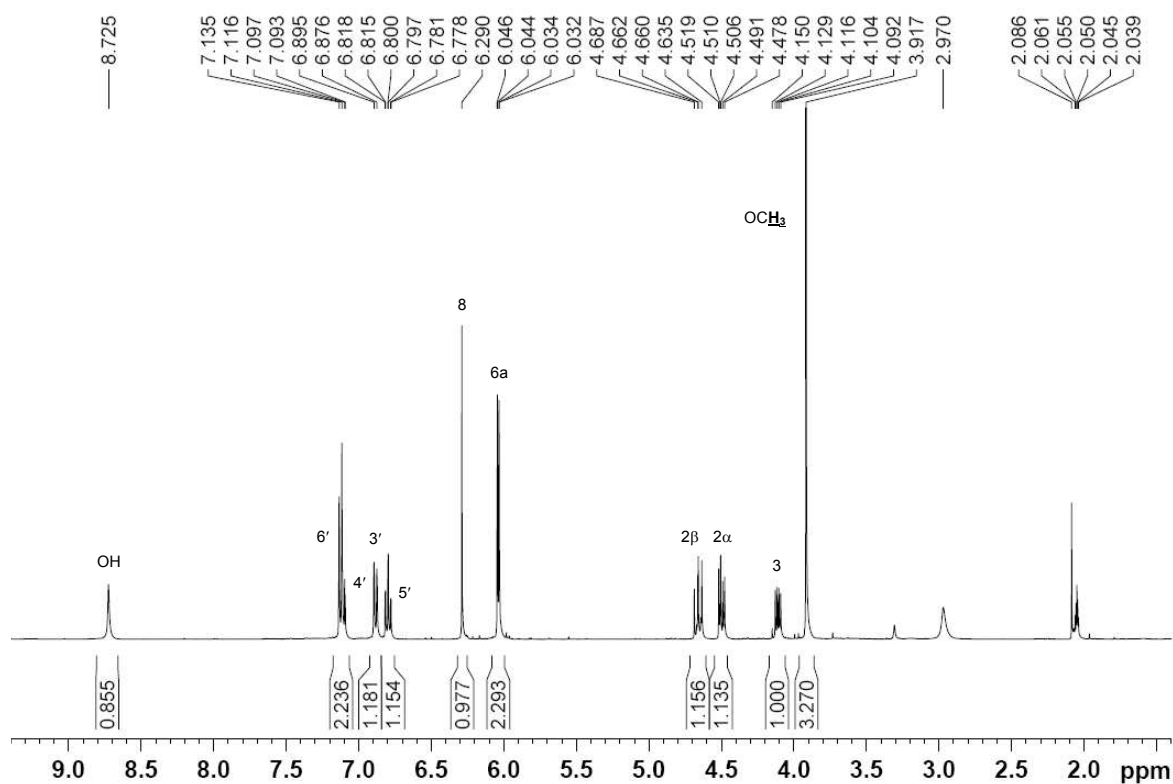


C.

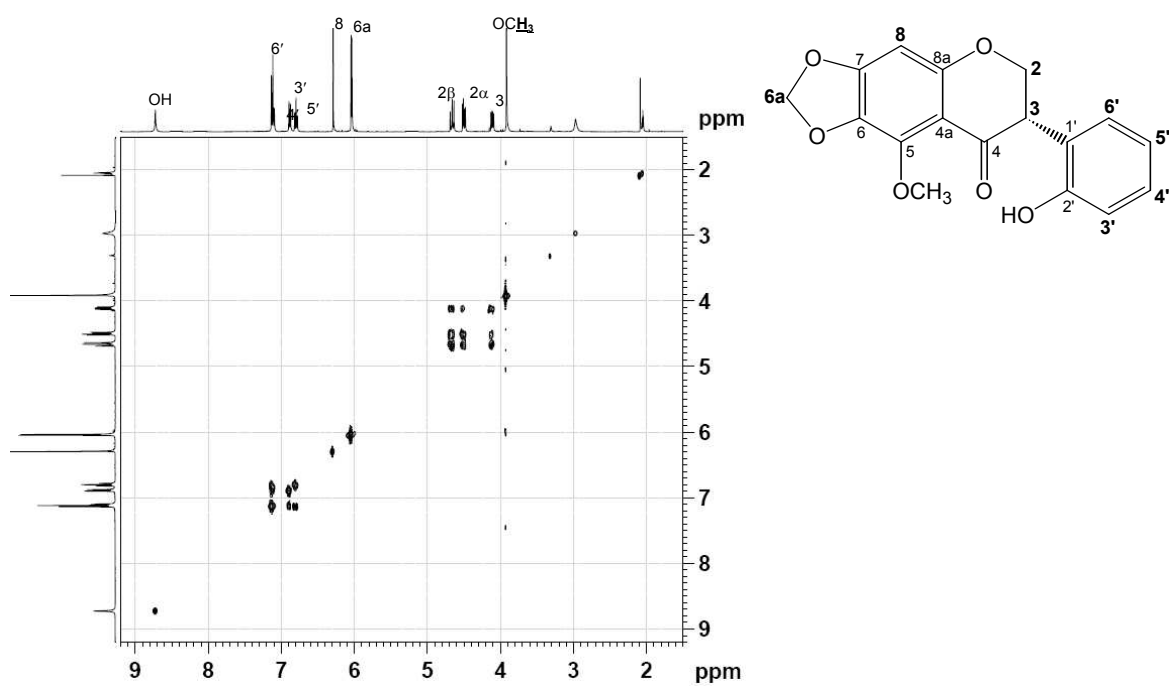


**Figure S11.** RP-HPLC spectrum (A) with DAD detection and LC-MS analysis (B) for **3**. (C) The UV spectrum from the DAD data.

A.



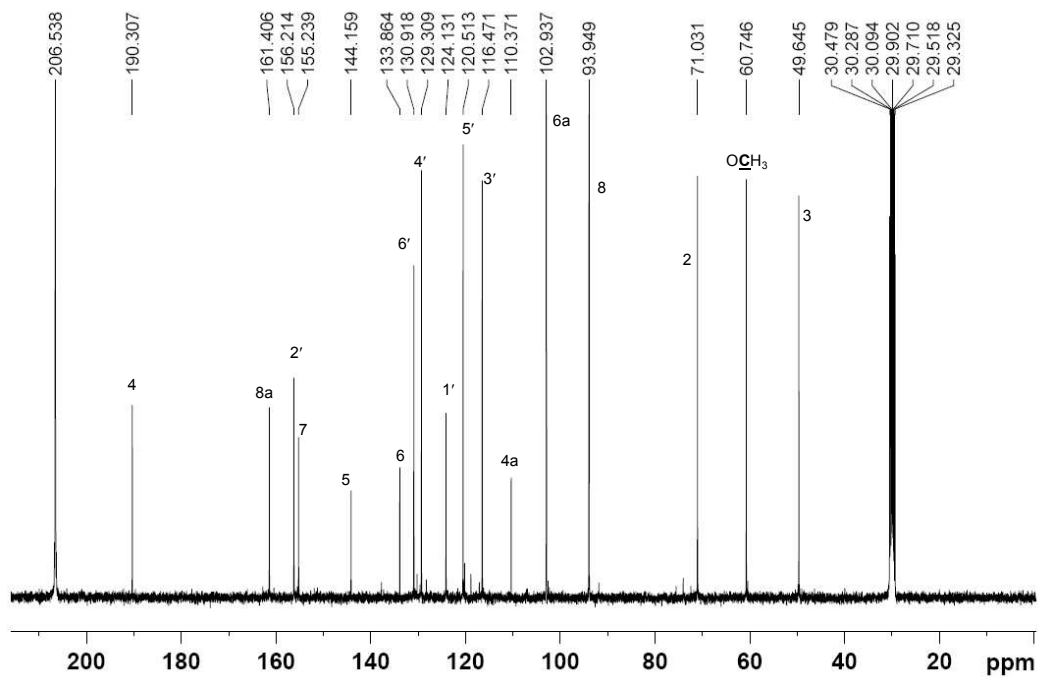
B.



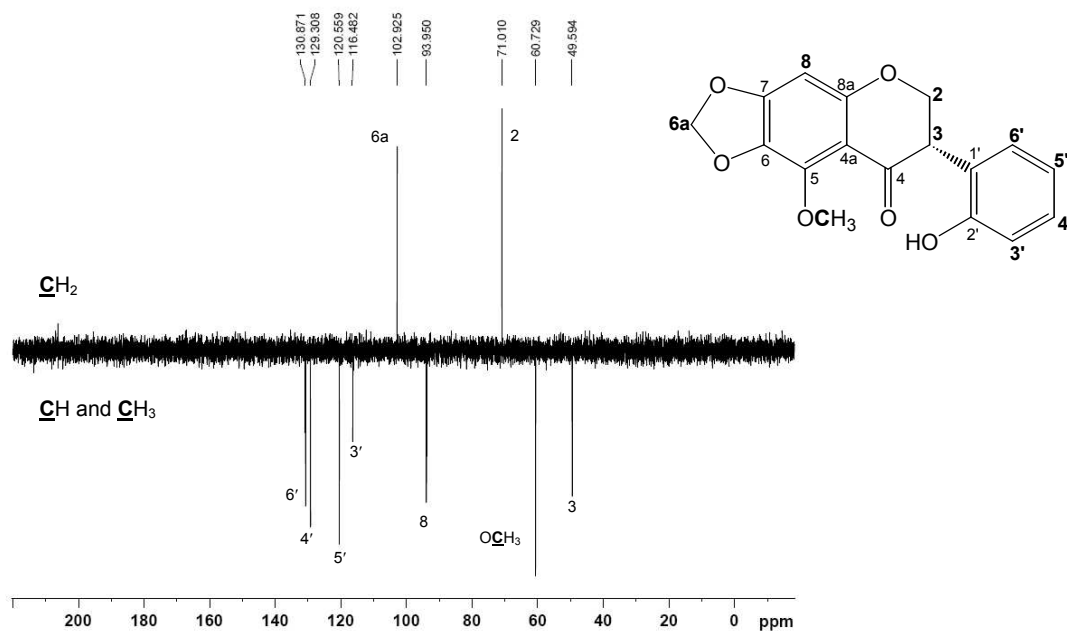
**Figure S12.** <sup>1</sup>H NMR spectrum (A) and <sup>1</sup>H-<sup>1</sup>H COSY spectrum (B) for **3** (400 MHz, acetone-d<sub>6</sub>).



A.



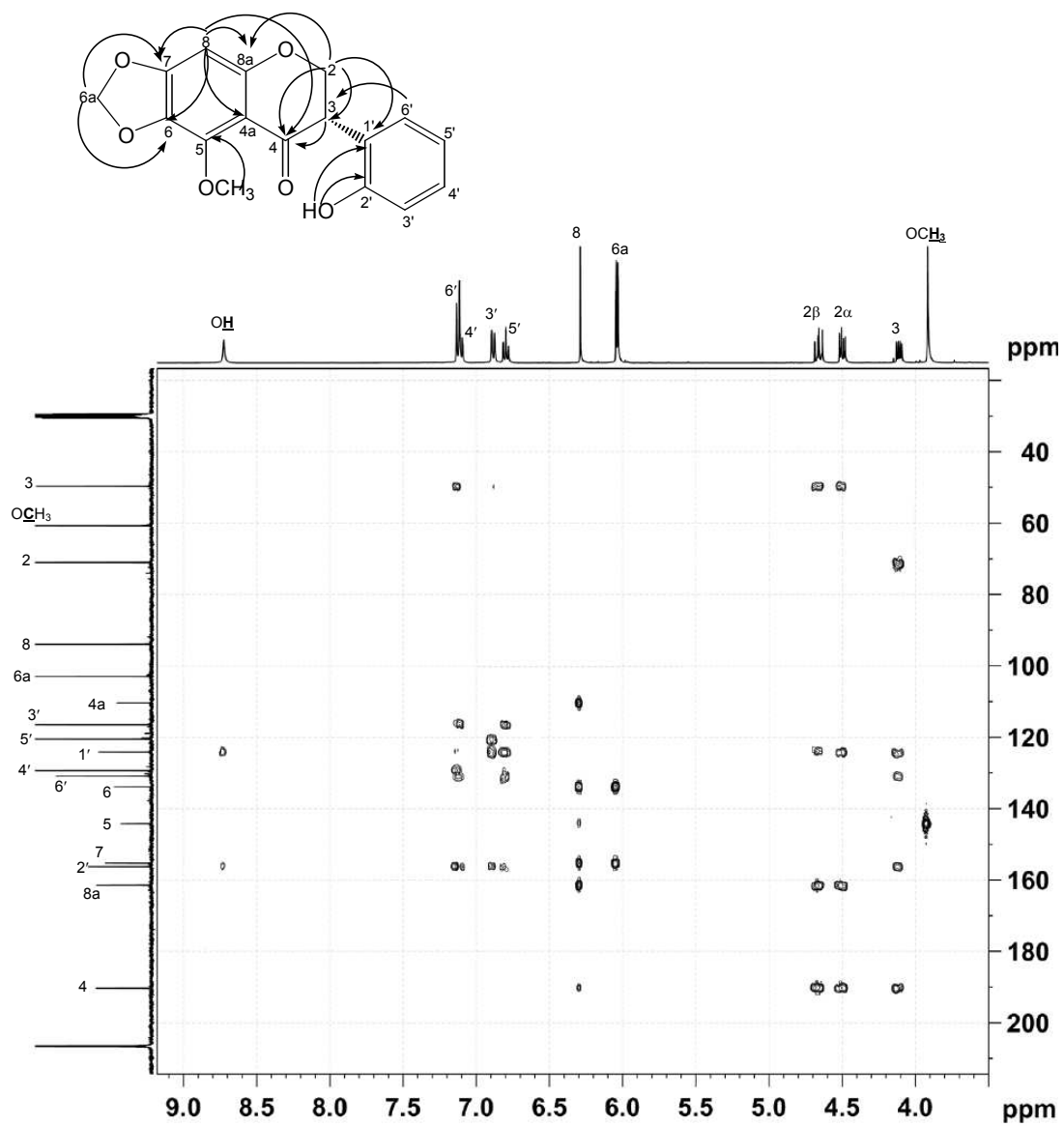
B.



**Figure S13.** <sup>13</sup>C NMR spectrum (A) and DEPT 135 spectrum (B) for **3** (100 MHz, acetone-d<sub>6</sub>).



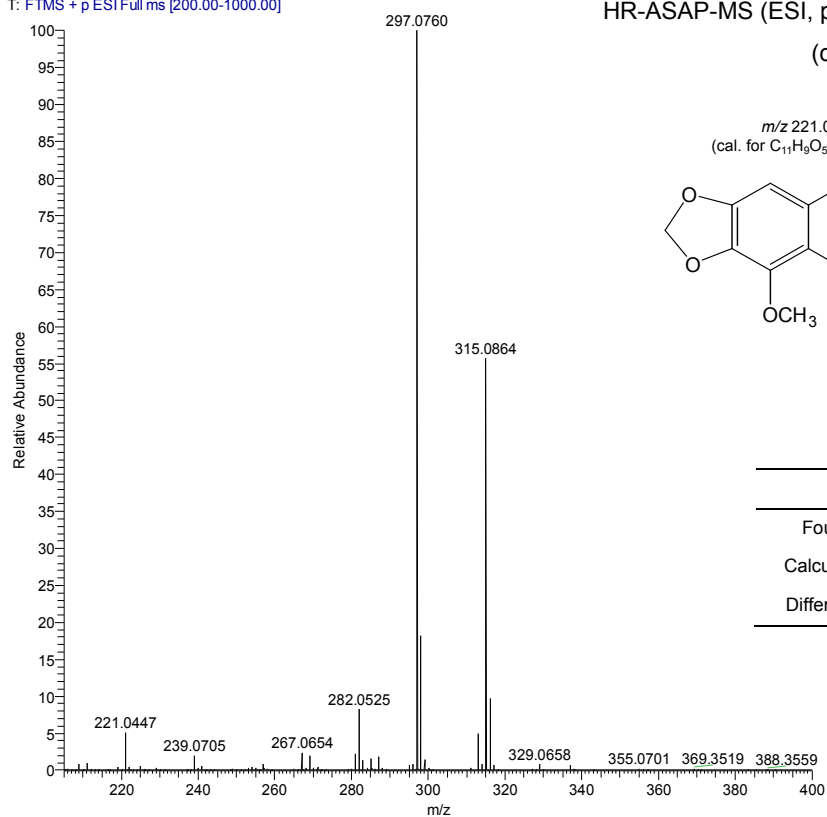
**Figure S14.** HMQC spectrum for **3** (100 MHz, acetone-d<sub>6</sub>).



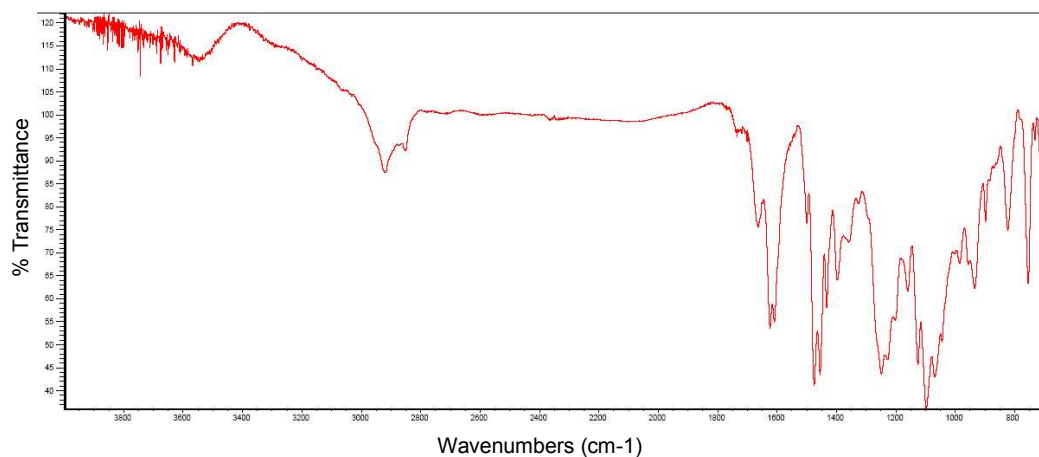
**Figure S15.** HMBC spectrum for **3** (100 MHz, acetone-d<sub>6</sub>). Major interactions are indicated in the compound structure.

A.

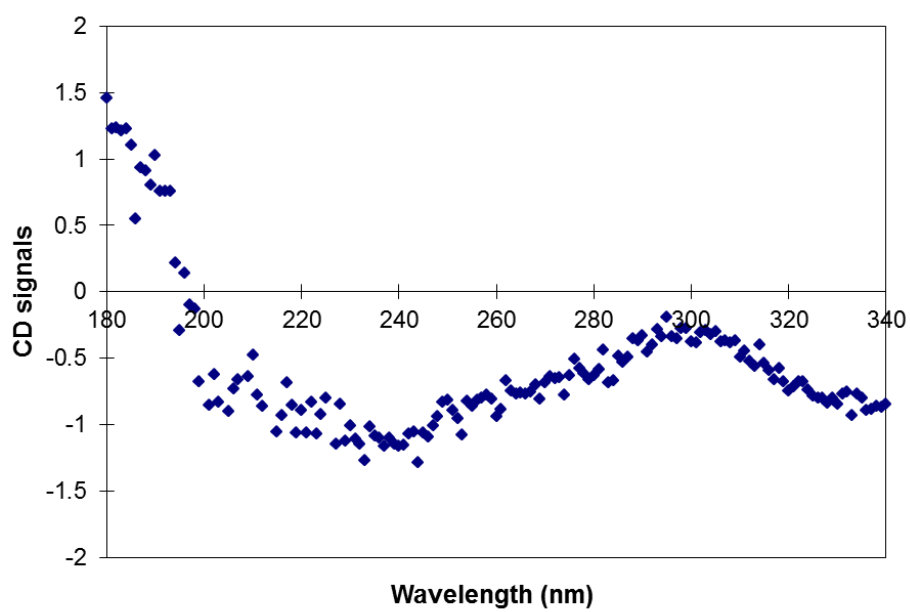
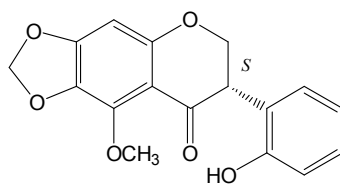
ip245-3 #17 RT: 0.24 AV: 1 NL: 3.98E8  
T: FTMS + p ESI Full ms [200.00-1000.00]



B.

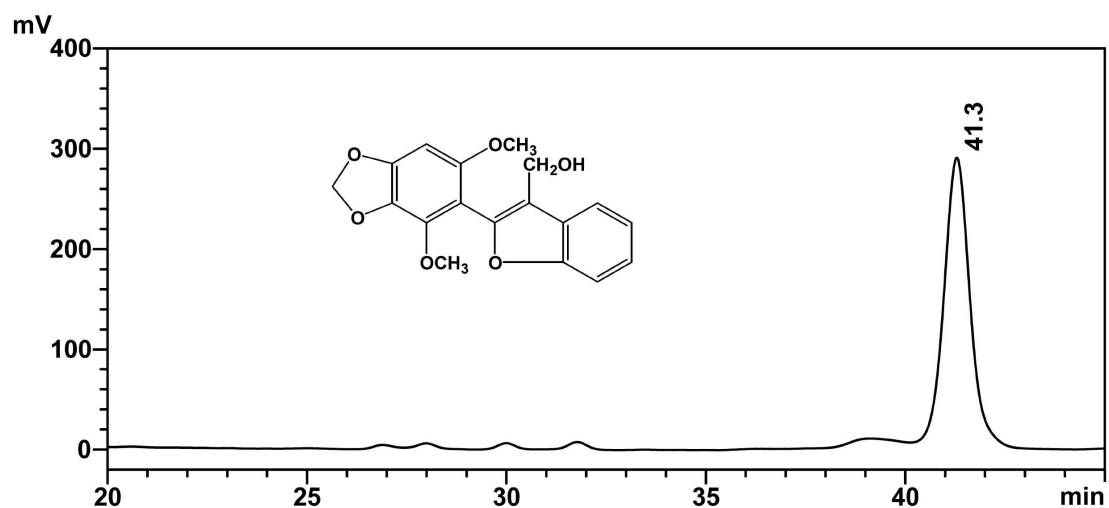


**Figure S16.** HRMS spectrum (A) and FT-IR spectrum (B) for **3**.



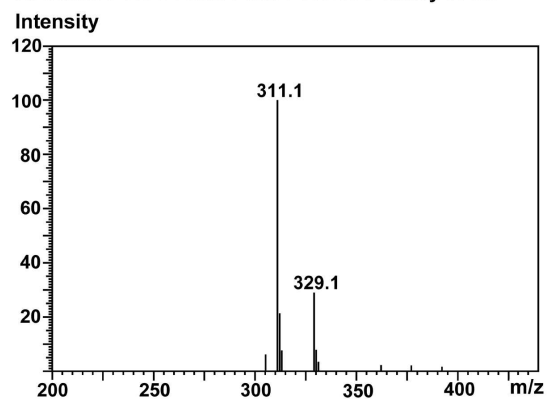
**Figure S17.** CD spectrum for **3**.

A.

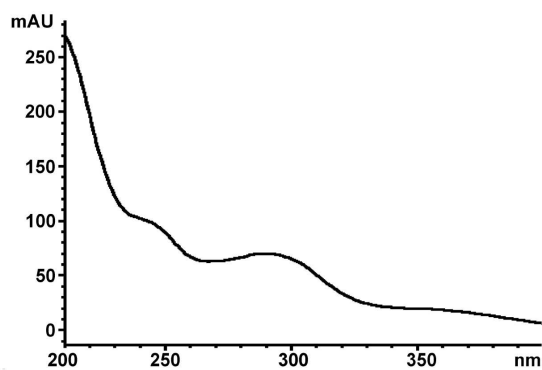


B.

Ret.Time : 41.2 / Base Peak : 311.1 / Polarity : Pos

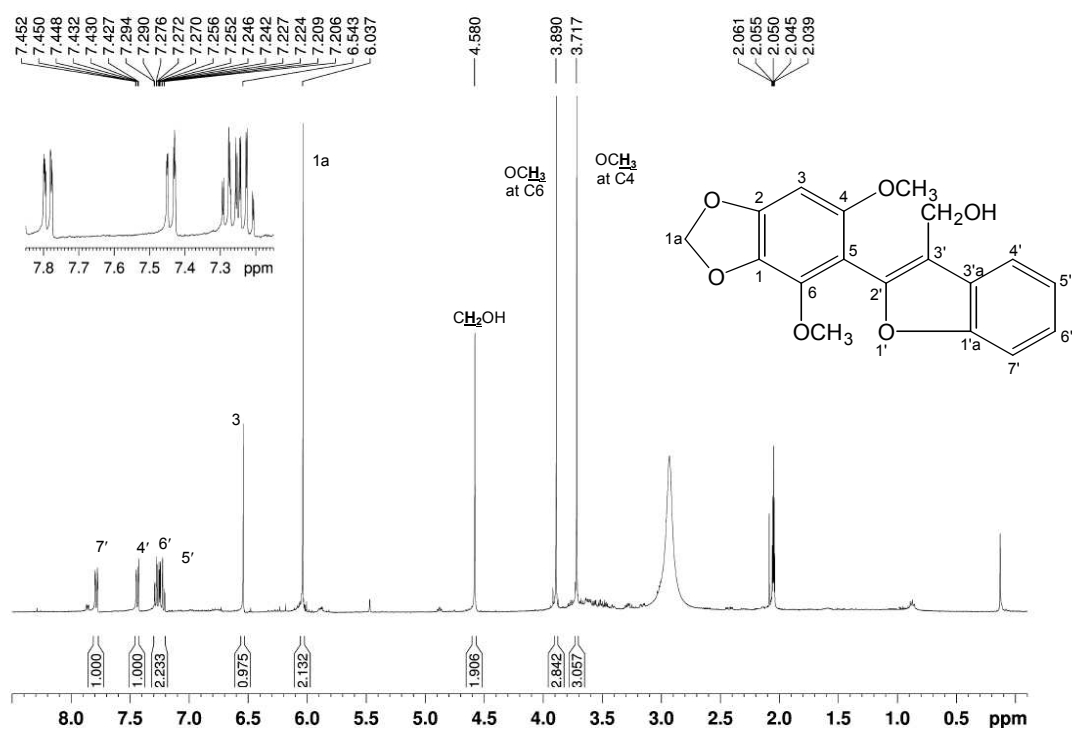


C.

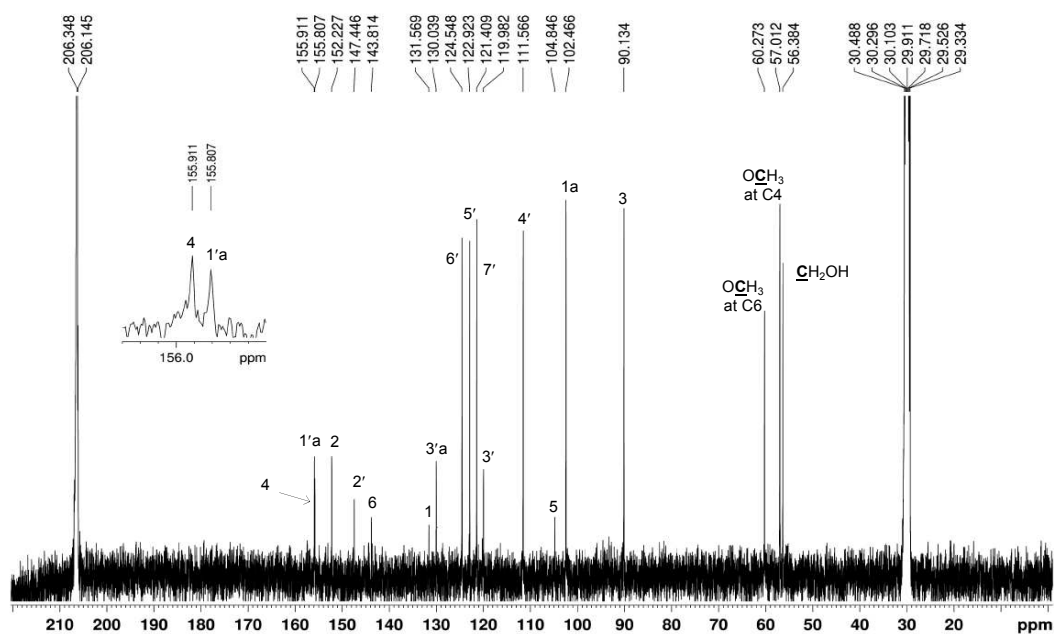


**Figure S18.** RP-HPLC spectrum (A) with DAD detection and LC-MS analysis (B) for **4**. (C) The UV spectrum from the DAD data.

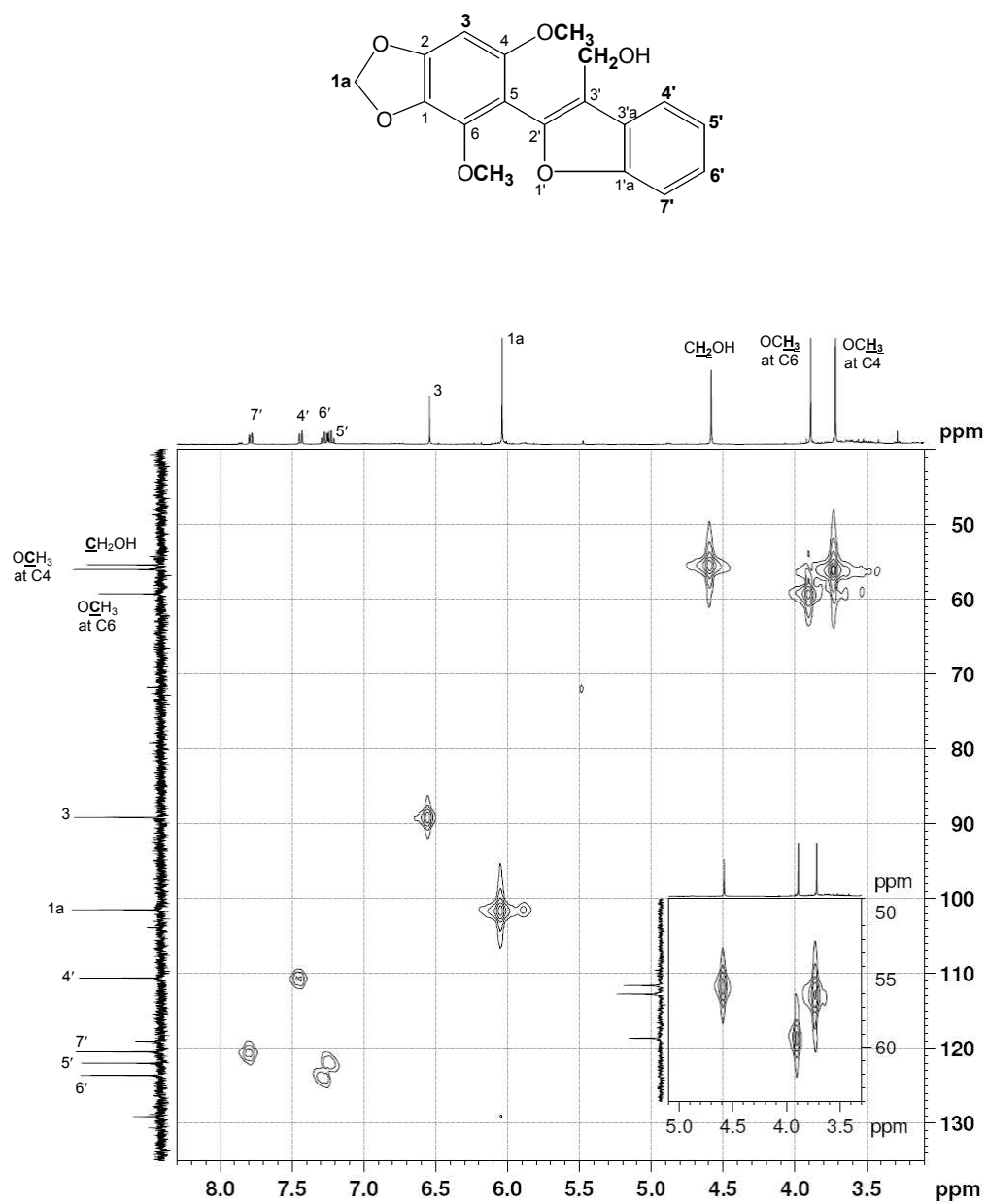
A.



B.



**Figure S19.** <sup>1</sup>H NMR spectrum (400 MHz, acetone-d<sub>6</sub>) (A) and <sup>13</sup>C NMR spectrum (100 MHz, acetone-d<sub>6</sub>) (B) for 4.



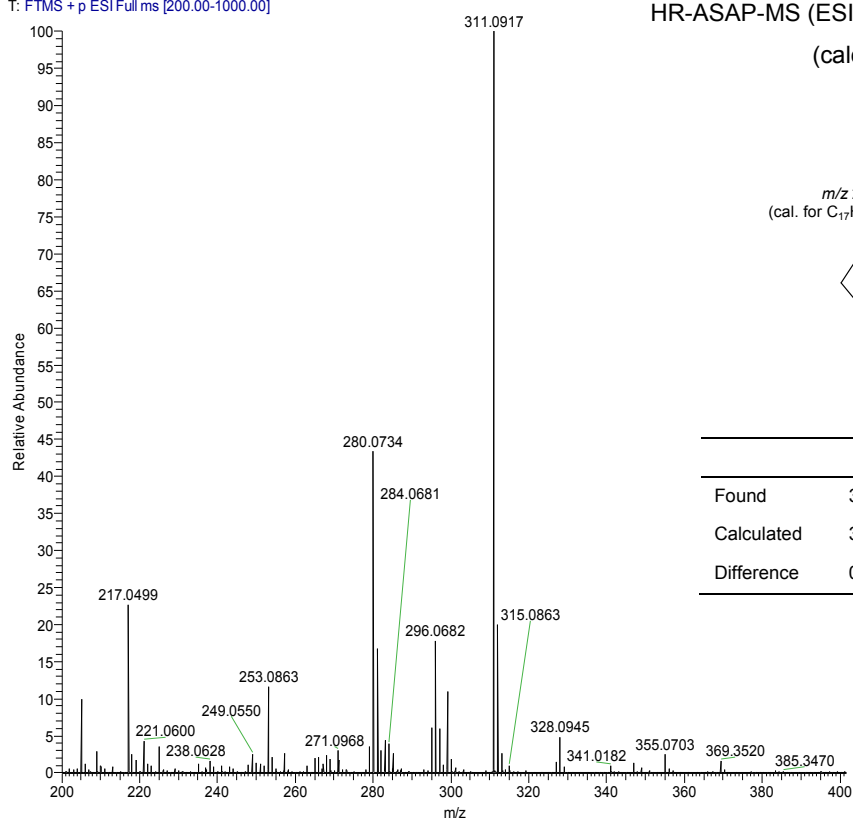
**Figure S20.** HMQC spectrum for **4** (100 MHz, acetone-d<sub>6</sub>).



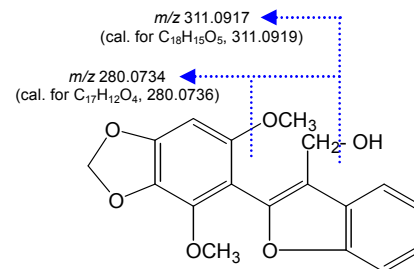


A.

jp245-5 #4 RT: 0.06 AV: 1 NL: 5.22E7  
T: FTMS + p ESI Full ms [200.00-1000.00]



HR-ASAP-MS (ESI, positive ion,  $m/z$  328.0945  $[M]^+$ )  
(calculated for  $C_{18}H_{16}O_6$ , 328.0947)



	$[M]^+$	$[M-OH]^+$	$[M-OH-CH_3]^+$
Found	328.0945	311.0917	280.0734
Calculated	328.0947	311.0919	280.0736
Difference	0.61 ppm	0.64 ppm	0.71 ppm

B.

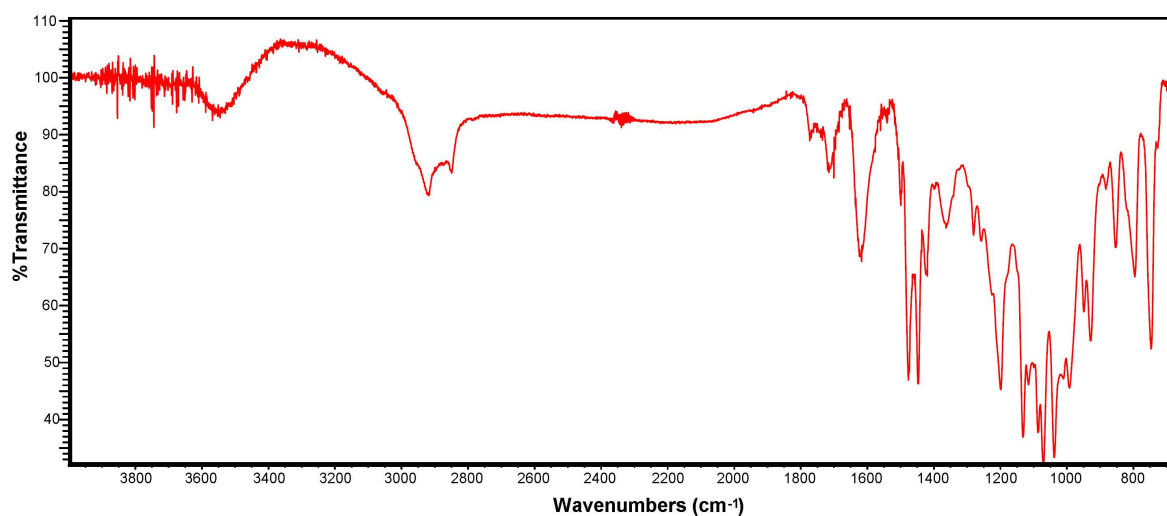
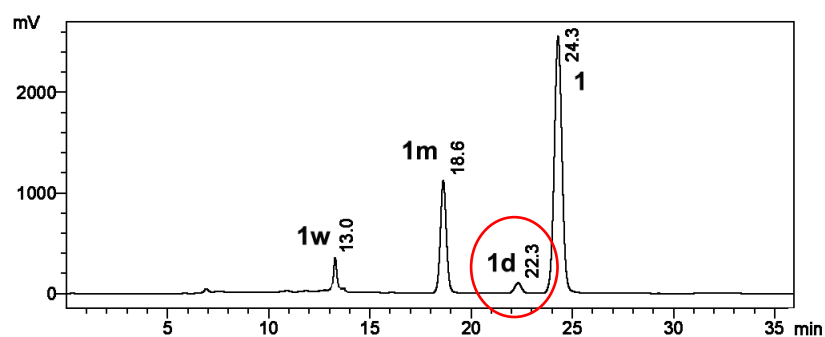
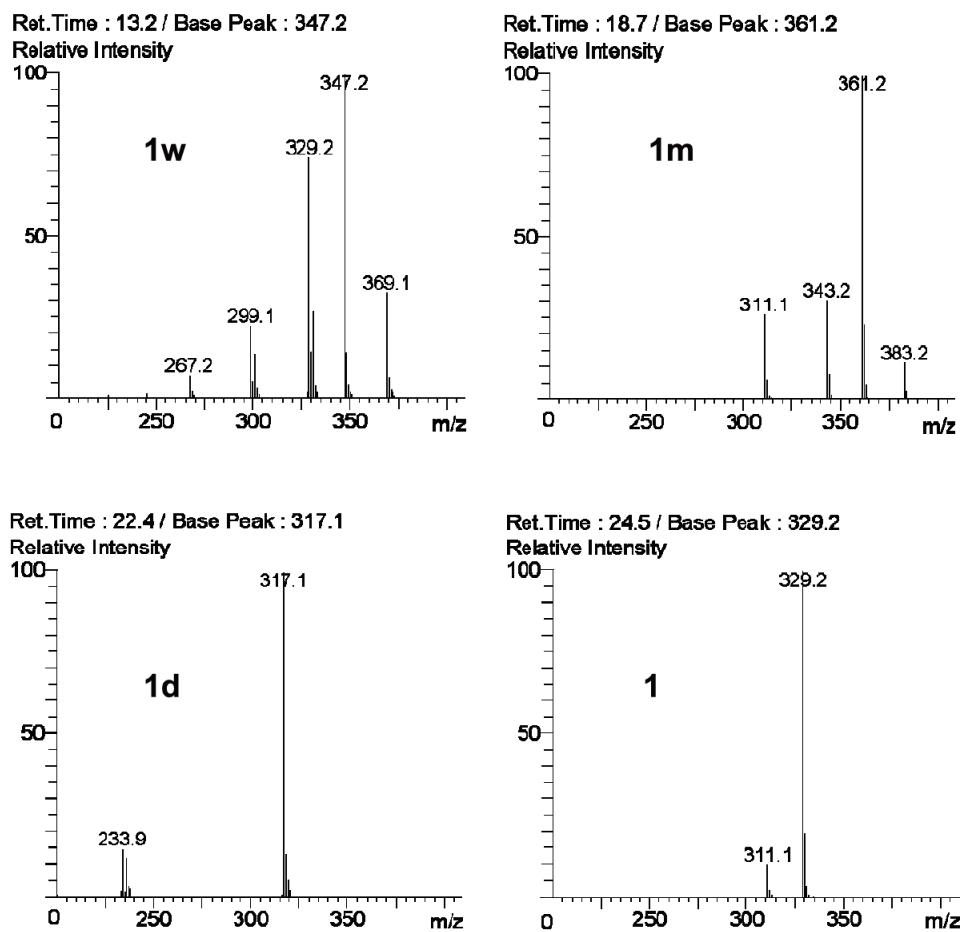


Figure S22. HRMS spectrum (A) and FT-IR spectrum (B) for 4.

A.

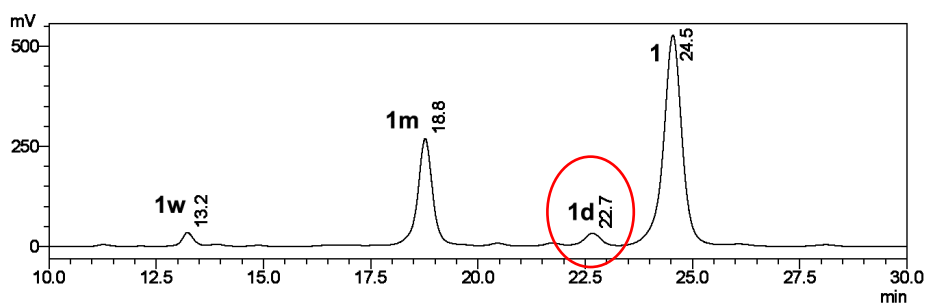


B.

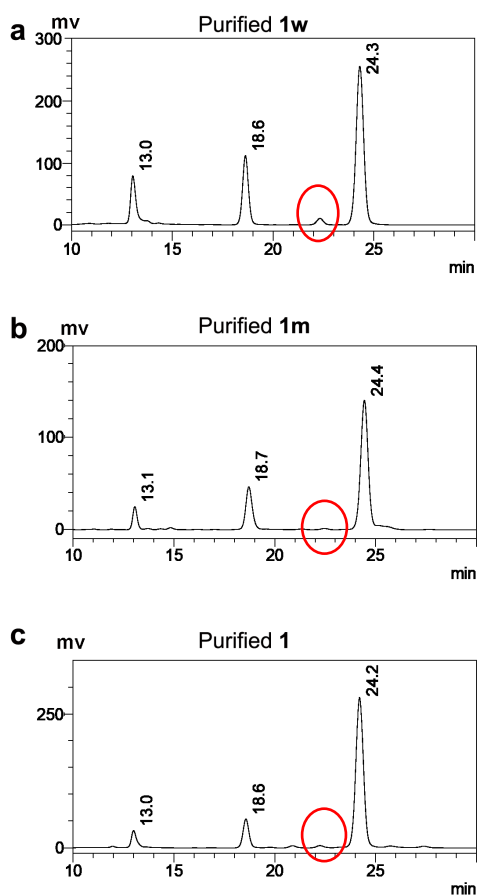


**Figure S23.** LC-MS (ESI, positive mode,  $m/z$ ) analysis of the water adduct **1w**, MeOH adduct **1m**, **1**, and **1d** in the water/MeOH/ACN (4:3:3, v/v/v) mobile phase. (A) LC chromatogram with retention times of the compound peaks. (B) MS analysis for each peak shown in the LC chromatogram.

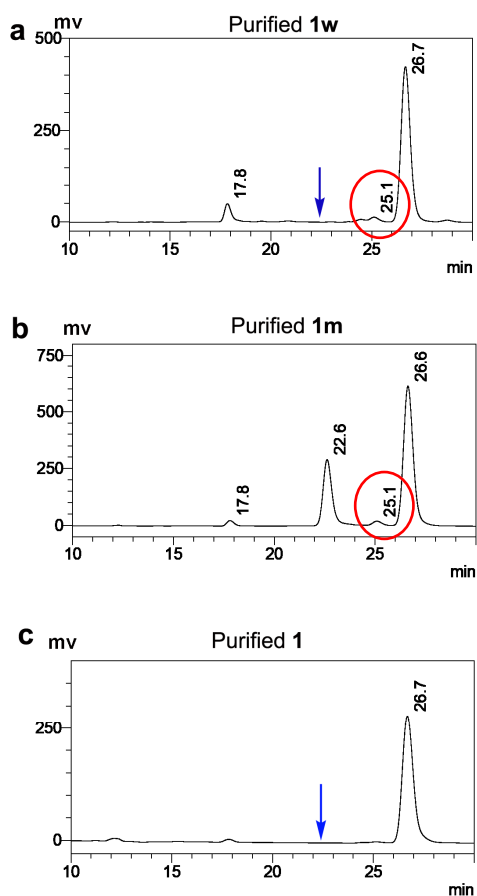
A.



B.

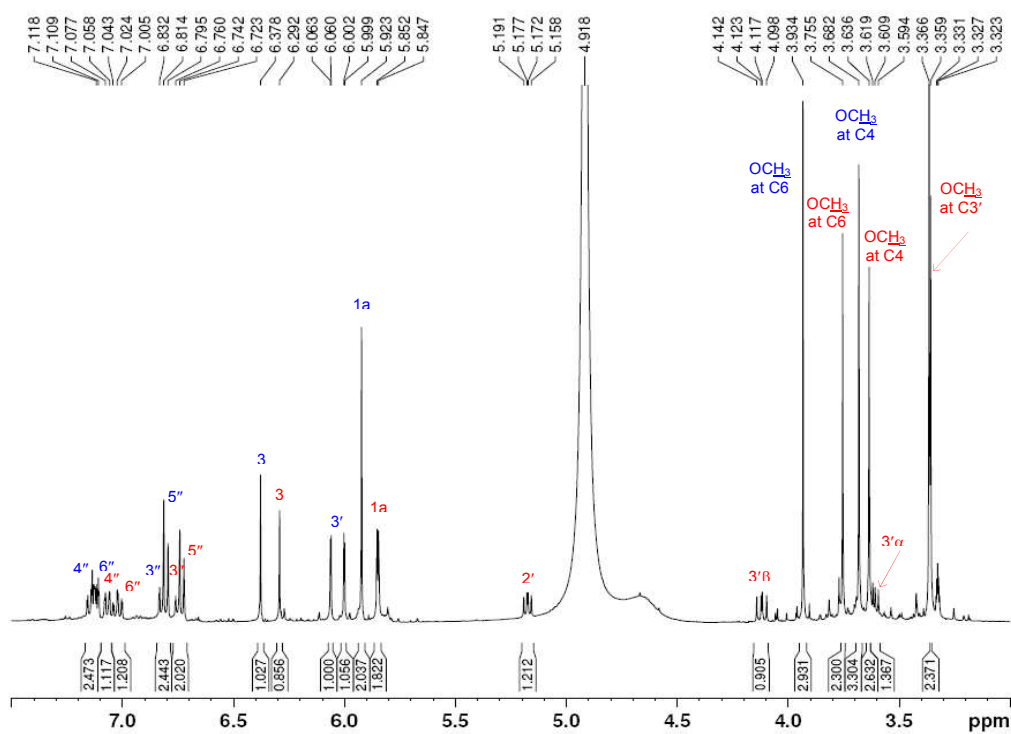


C.

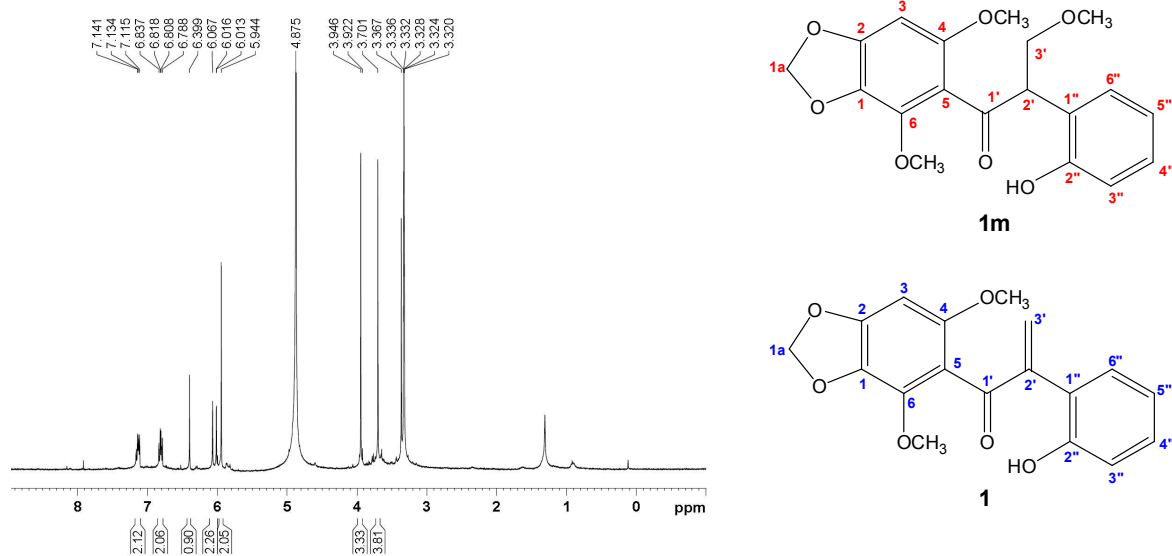


**Figure S24.** RP-HPLC analysis of the water adduct **1w** (a), MeOH adduct **1m** (b), and **1** (c) purified in the EtOH-based solvent. (A) LC chromatogram prior to purification of all compounds. (B) LC chromatogram analyzed in the water/MeOH/ACN (4:3:3, v/v/v) mobile phase with each of the purified compounds. (C) LC chromatograms analyzed in the water/EtOH/ACN (4:3:3, v/v/v) mobile phase with each of the purified compounds. The blue arrows are the positions for **1m**, which was detected only in the use of MeOH-based solvent. The red circles are for **1d**.

**A.**

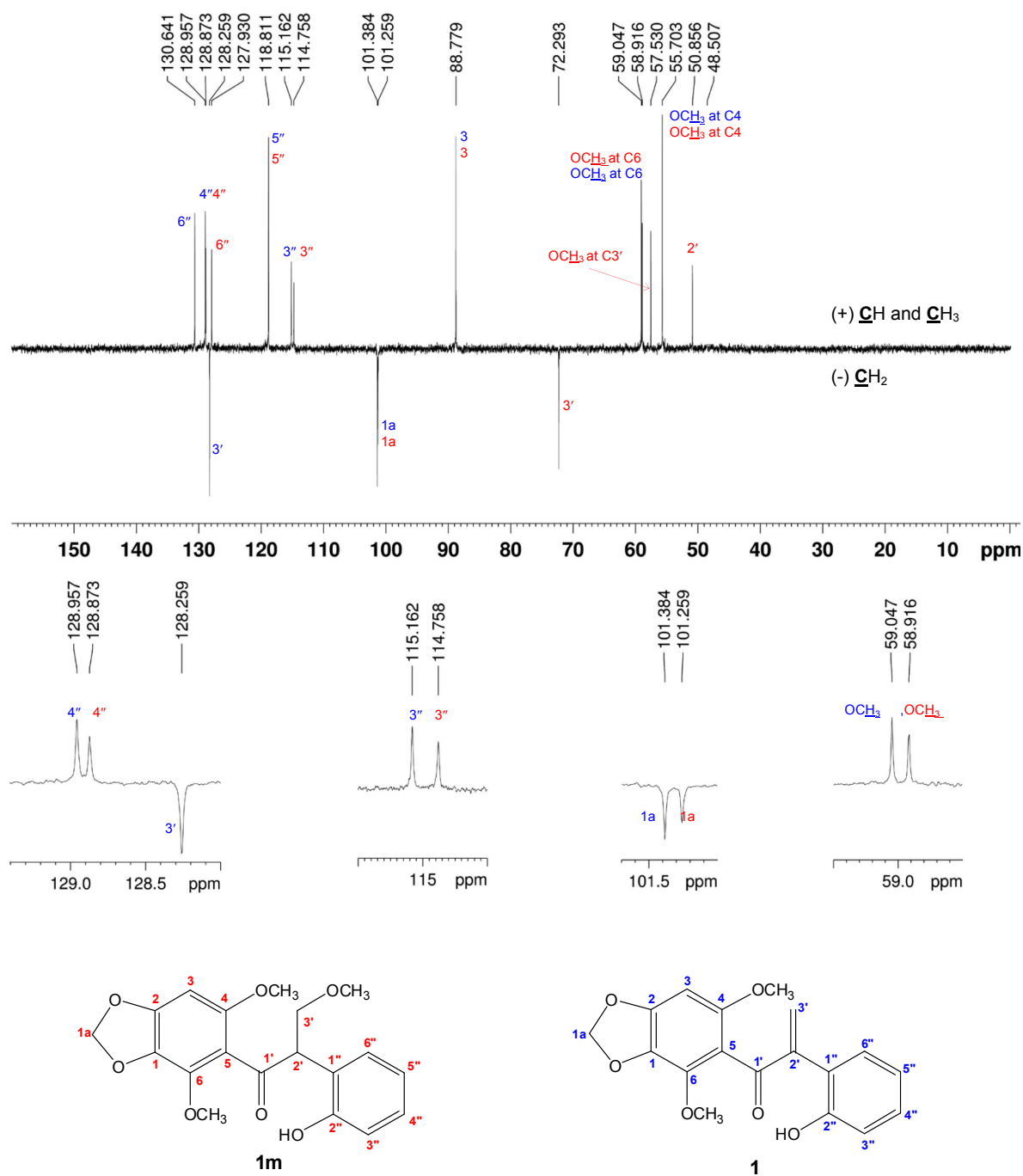


**B.**

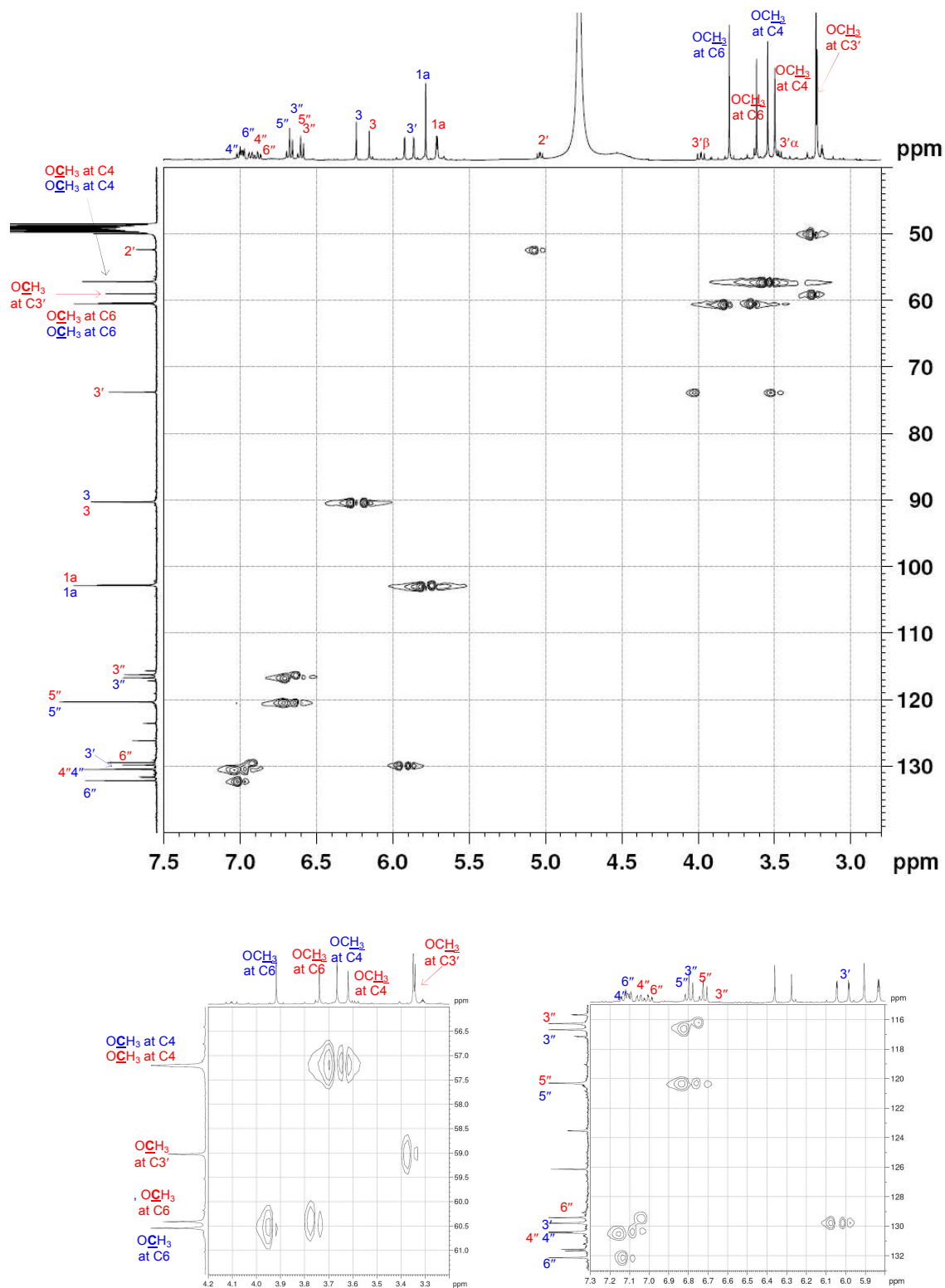


**Figure S25.**  $^1\text{H}$  NMR spectrum for MeOH adduct **1m** and **1** (400 MHz,  $\text{CD}_3\text{OD}$ ). The  $\text{CD}_3\text{OD}$  lock solvent was used to maximize the yield for the NMR experiment. (A)  $^1\text{H}$  NMR spectrum of a mixture of the MeOH adduct **1m** (red) and **1** (blue). (B)  $^1\text{H}$  NMR spectrum for **1** purified in the EtOH-based RP-HPLC (400 MHz,  $\text{CD}_3\text{OD}$ ).



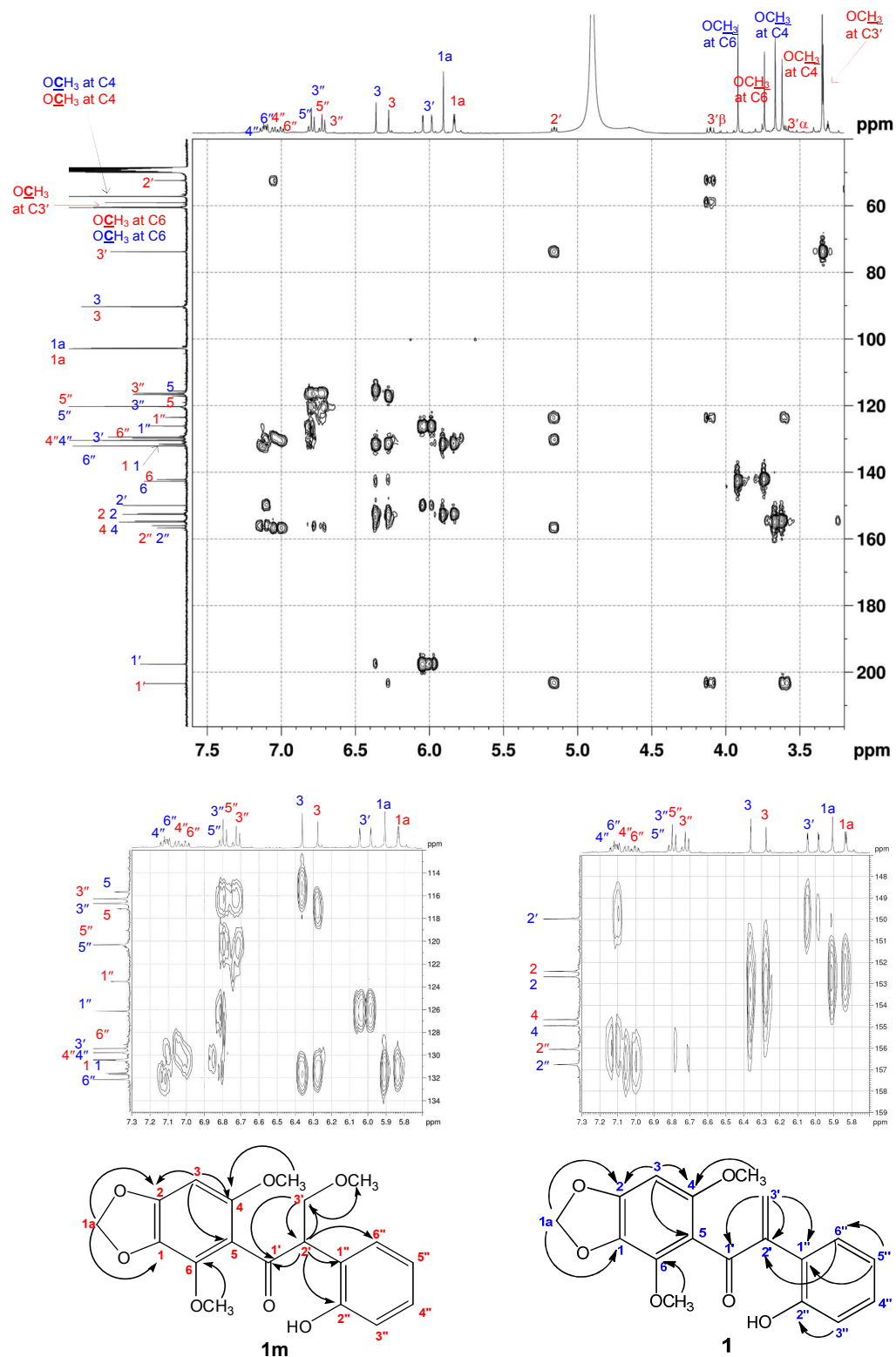


**Figure S27.** DEPT135 spectrum for a mixture of the MeOH adduct **1m** (red) and **1** (blue) (100 MHz, CD<sub>3</sub>OD).



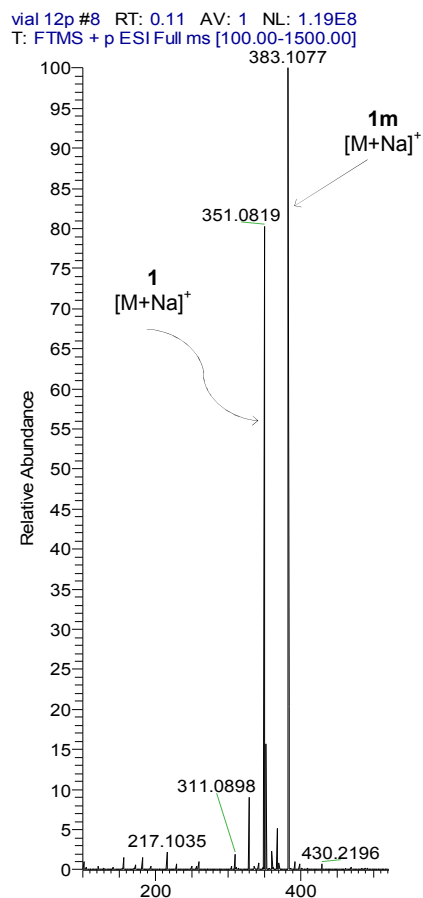
**Figure S28.** HSQC spectrum for a mixture of the MeOH adduct **1m** (red) and **1** (blue)(100 MHz, CD<sub>3</sub>OD).



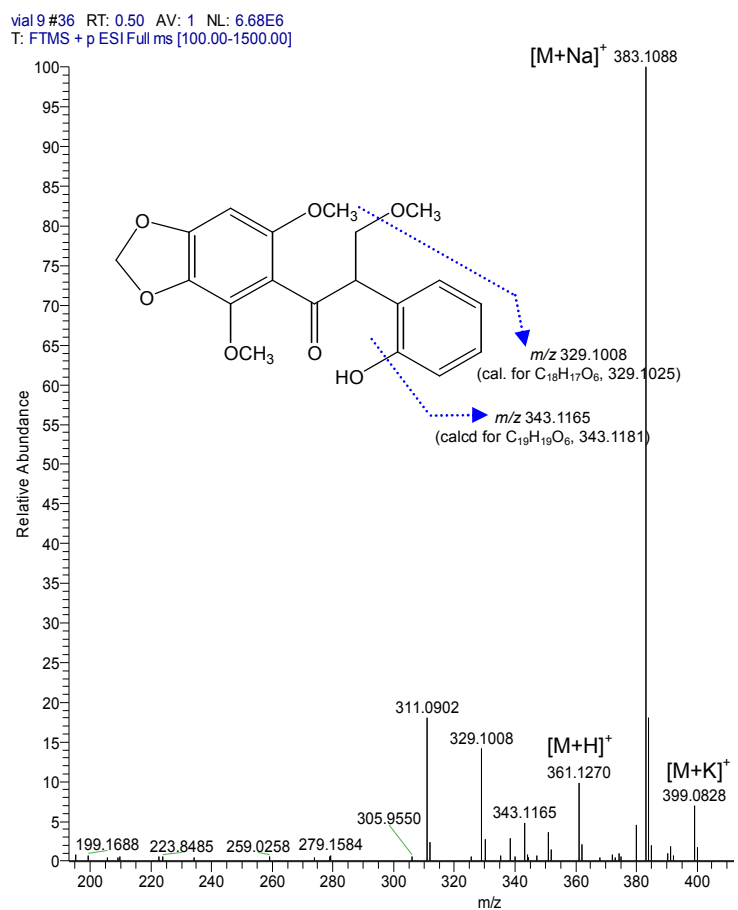


**Figure S29.** HMBC spectra for a mixture of the MeOH adduct **1m** (red) and **1** (blue) (100 MHz, CD<sub>3</sub>OD). Major interactions are indicated in the compound structures.

A.

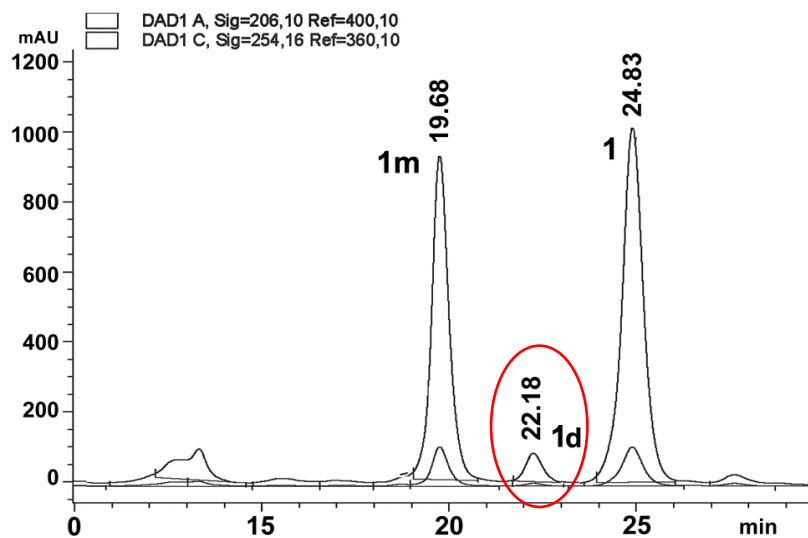
Compound **1m**HRMS (ESI, positive mode, *m/z*)383.1077 [M+Na]<sup>+</sup>(calculated for C<sub>19</sub>H<sub>20</sub>NaO<sub>7</sub>, 383.1107)Compound **1**HRMS (ESI, positive mode, *m/z*)351.0819 [M+Na]<sup>+</sup>(calculated for C<sub>18</sub>H<sub>16</sub>NaO<sub>6</sub>, 351.0844)

B.

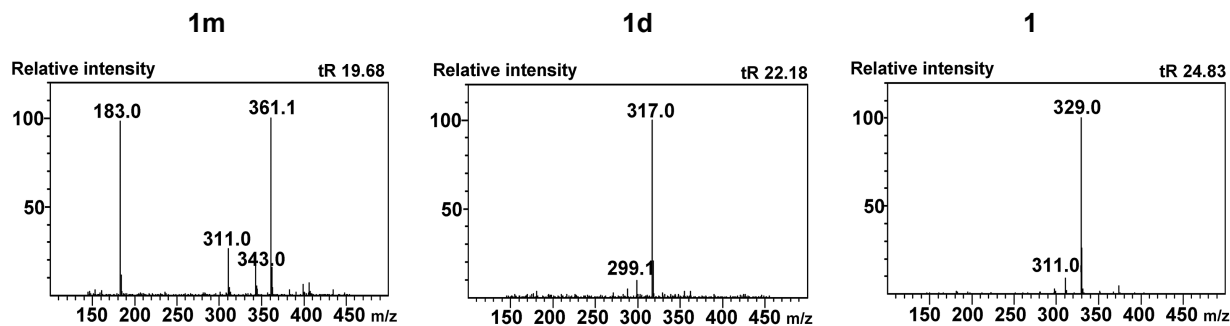
Compound **1m**HRMS (ESI, positive mode, *m/z*)361.1270 [M+H]<sup>+</sup>(calculated for C<sub>19</sub>H<sub>21</sub>O<sub>7</sub>, 361.1287)383.1088 [M+Na]<sup>+</sup>(calculated for C<sub>19</sub>H<sub>20</sub>NaO<sub>7</sub>, 383.1107)399.0828 [M+K]<sup>+</sup>(calculated for C<sub>19</sub>H<sub>20</sub>KO<sub>7</sub>, 399.0846)

**Figure S30.** HRMS spectrum of a mixture of **1m** and **1**. (A) MS spectrum for a mixture of **1m** and **1** in MeOH carrier solvent. (B) MS spectrum for **1m**, the MeOH adduct, extracted from (A) spectrum data.

A.

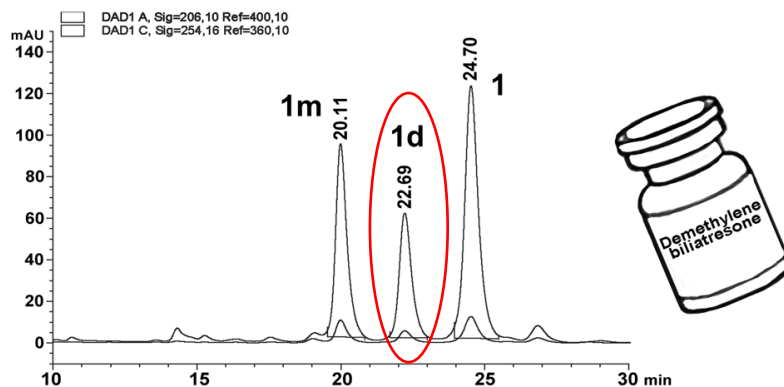


B.

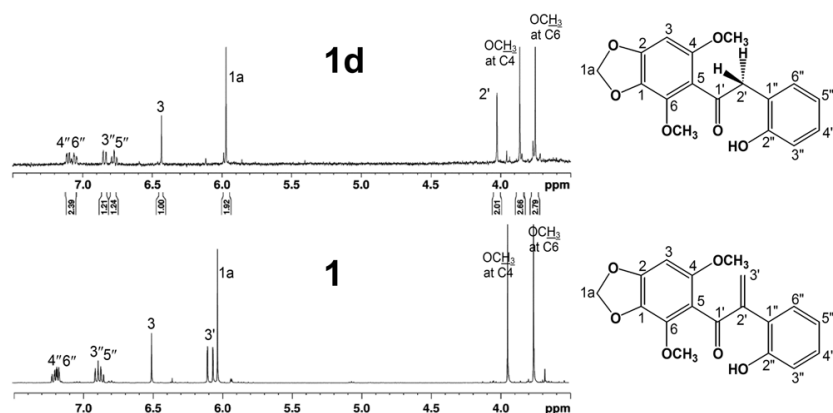


**Figure S31.** LC-MS (ESI, positive mode,  $m/z$ ) analysis of the minor peak **1d** (red open circle) with **1m** and **1**. (A) LC chromatogram in water/ACN (1:1, v/v) mobile phase and flow rate  $0.3 \text{ mL} \cdot \text{min}^{-1}$ . (B) MS analysis of the LC chromatogram: **1m** ( $t_R$  19.68 min), **1d** ( $t_R$  22.18 min), and **1** ( $t_R$  24.83 min). The minor peak at  $t_R$  22.18 min was collected to identify the chemical structure of **1d**.

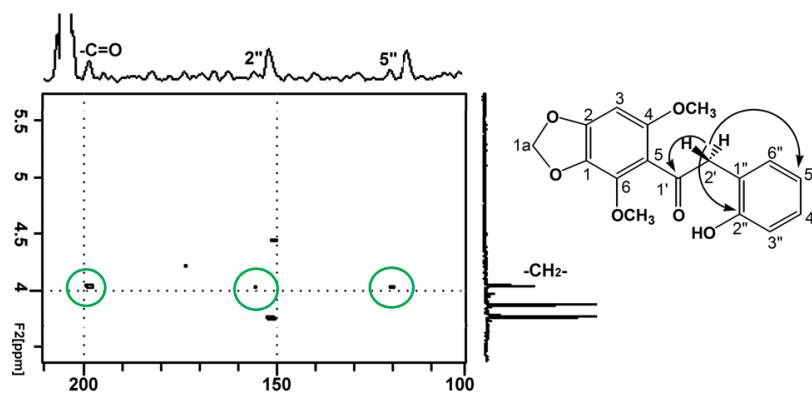
A.



B.

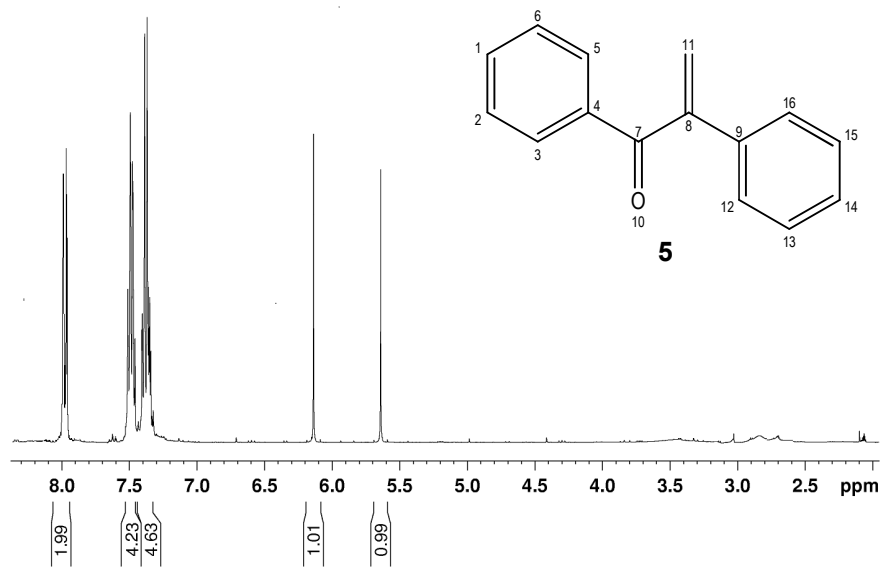


C.

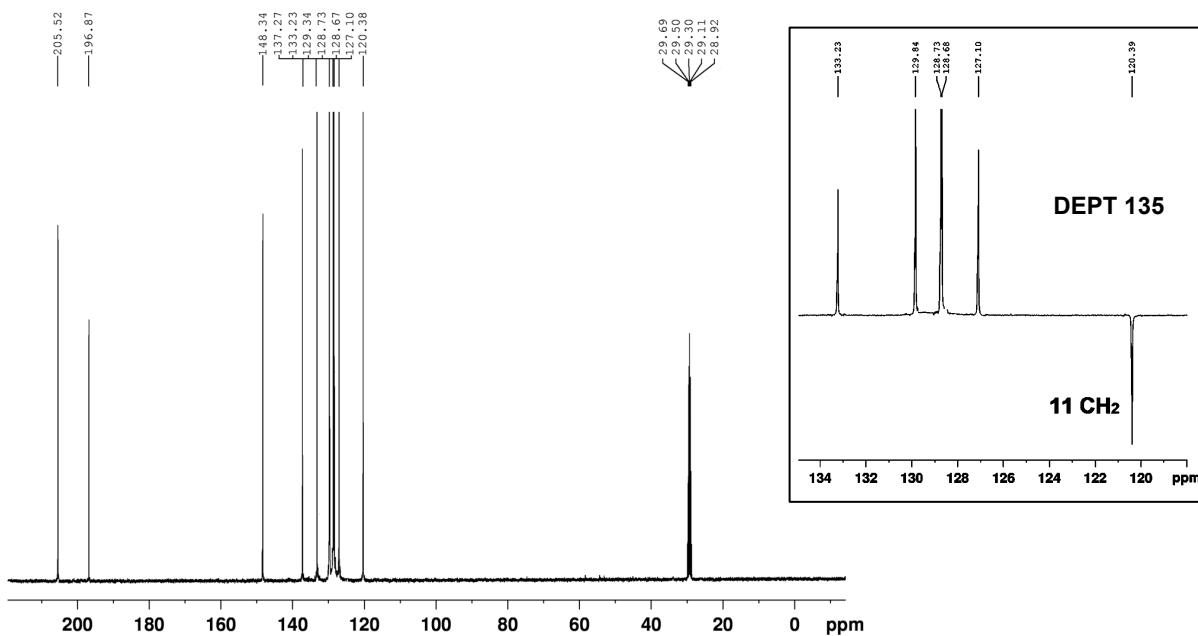


**Figure S32.** Purification and identification of the chemical structure of **1d**. (A) RP-HPLC chromatogram after conversion of **1d**, caused by addition of MeOH, to **1m** ( $t_R$  20.11 min) and **1** ( $t_R$  24.70 min). The peak **1d** ( $t_R$  22.69 min) was purified with the water/ACN solvent. (B)  $^1\text{H}$  NMR spectrum (400MHz, acetone- $d_6$ ) of the purified **1d** and comparison with that of **1**. (C) HMBC spectrum (100MHz, acetone- $d_6$ ) of the purified **1d**. Major HMBC correlations of the ethanone bridge ( $-\text{CH}_2-$ ) signals indicated with the green circles.

A.

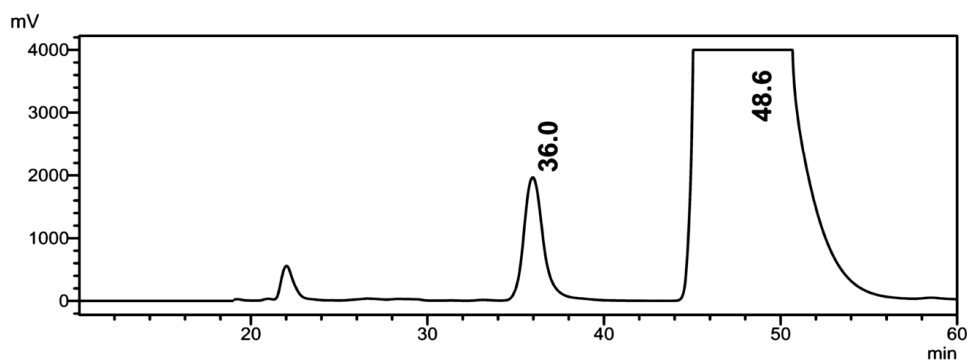


B.

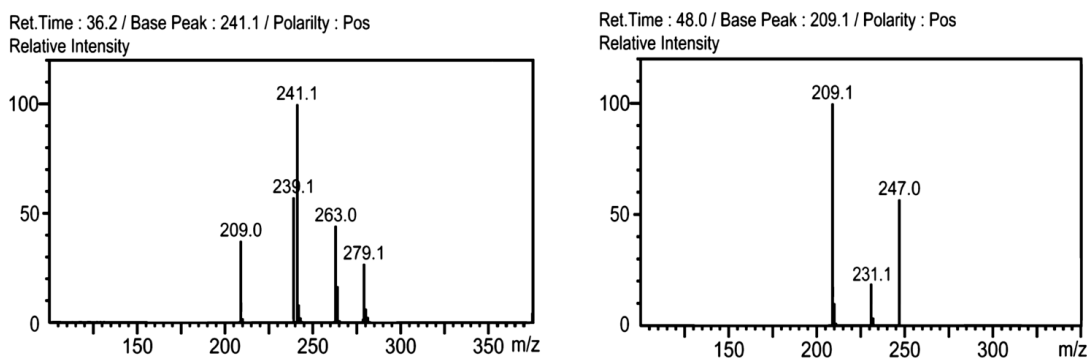


**Figure S33.** NMR spectra for the synthetic **5**. (A) <sup>1</sup>H NMR spectrum (400 MHz, acetone-d<sub>6</sub>) and (B) <sup>13</sup>C NMR and DEPT135 spectra (100 MHz, acetone-d<sub>6</sub>). The DEPT135 spectrum showed CH<sub>2</sub> peak of a methylene carbon (δ<sub>C</sub>=120.4 ppm, C11).

A.



B.



**Figure S34.** LC-MS (ESI, positive mode,  $m/z$ ) analysis of the synthetic **5**. (A) LC chromatogram of **5** in the water/MeOH/ACN solvent. Two peaks appeared at the retention times of  $t_R$  36.0 and  $t_R$  48.6 min, respectively. (B) MS analysis of the two peaks shown in the LC chromatogram. The peak at  $t_R$  36.0 showed a mass at  $m/z$  241  $[M+H]^+$ , 263  $[M+Na]^+$ , and 279  $[M+K]^+$ , indicating a molecular formula of  $C_{16}H_{16}O_2$ , (calc. 240.1150) formed by conjugation of MeOH ( $CH_3OH$ ; 32 amu), while the peak at  $t_R$  48.6 exhibited a mass at  $m/z$  209  $[M+H]^+$ , 231  $[M+Na]^+$ , and 247  $[M+K]^+$  corresponding to the molecular formula of  $C_{15}H_{12}O$  (calc. 208.0888) of **5**.

**Table S1.** 1D and 2D NMR data of compounds **1m** and **1**.

<b>1m<sup>a</sup></b>					<b>1<sup>a</sup></b>				<b>1<sup>b</sup></b>			
No.	$\delta_{\text{H}}$ ( <i>J</i> in Hz)	$\delta_{\text{C}}$		HMBC	$\delta_{\text{H}}$ ( <i>J</i> in Hz)	$\delta_{\text{C}}$		HMBC	$\delta_{\text{H}}$ ( <i>J</i> in Hz)	$\delta_{\text{C}}$		HMBC
1		131.6	C			131.9	C			131.5	C	
1a	5.85 d (2.0)	102.8	CH <sub>2</sub>	1, 2	5.92 s	102.9	CH <sub>2</sub>	1, 2	6.01 s	102.5	CH <sub>2</sub>	1, 2
2		152.4	C			152.7	C			151.9	C	
3	6.29 s	90.3	CH	1. 2, 5, 6, 1'	6.38 s	90.3	CH	1. 2, 5, 6, 1'	6.48 s	90.4	CH	1, 2, 4, 5
4		154.7	C			154.9	C			154.1	C	
5		117.1	C			115.7	C			115.8	C	
6		142.2	C			142.7	C			142.0	C	
1'		203.4	C			197.6	C			195.7	C	
2'	5.17 dd (7.6, 5.6)	52.4	CH	1', 3', 1'', 2'', 6''		149.9	C			149.5	C	
3'	4.12 dd (7.6, 10)	73.8	CH <sub>2</sub>	1', 2', 1'', OCH <sub>3</sub>	6.00 d (1.2)	129.8	CH <sub>2</sub>	1', 2', 1''	6.04 d (0.8, 16)	130.1	CH <sub>2</sub>	1', 2', 1''
	3.61 dd (5.6, 10)			1', 2', 1''	6.06 d (1.2)				6.08 d (1.2, 16)			
1''		123.5	C			126.1	C			125.9	C	
2''		156.8	C			156.1	C			155.5	C	
3''	6.72 m	116.3	CH	2'', 5''	6.81 m	116.7	CH	2'', 5''	6.88 t (8)	116.9	CH	2'', 5''
4''	7.07 t (7.6)	130.4	CH	2'', 6''	7.12 m	130.5	CH	2'', 6''	7.17 t (8)	130.2	CH	2'', 6''
5''	6.73 t (7.6)	120.3	CH	1'', 3''	6.81 t (7.6)	120.3	CH	1'', 3'', 4''	6.83 t (7.6)	120.3	CH	1'', 3''
6''	7.02 t (7.6)	129.4	CH	2'', 4''	7.11 m	132.1	CH	2', 2'', 4''	7.15 m	131.9	CH	2', 2'', 4''
	3.36 s	59.0	OCH <sub>3</sub>	3'	3.68 s	57.2	OCH <sub>3</sub>	4	3.73 s	57.2	OCH <sub>3</sub>	4
	3.64 s	57.2	OCH <sub>3</sub>	4	3.93 s	60.5	OCH <sub>3</sub>	6	3.92 s	60.4	OCH <sub>3</sub>	6
	3.76 s	60.4	OCH <sub>3</sub>	6					8.05 s		OH	1'', 3''

Spectra were recorded at 400 MHz for <sup>1</sup>H NMR and 100 MHz for <sup>13</sup>C NMR. <sup>a</sup> measured in CD<sub>3</sub>OD; <sup>b</sup> measured in acetone-d<sub>6</sub>.

**Table S2.** 1D and 2D NMR data of compounds **3** and **4**.

<b>3</b>					<b>4</b>				
No.	$\delta_{\text{H}}$ ( <i>J</i> in Hz)	$\delta_{\text{C}}$		HMBC	No.	$\delta_{\text{H}}$ ( <i>J</i> in Hz)	$\delta_{\text{C}}$		HMBC
2	4.66 dd (9.6, 10.8) 4.51 dd (5.2, 10.8)	71.0	CH <sub>2</sub>	3, 4, 8a, 1'	1		131.6	C	
3	4.11 d (5.2)	49.7	CH	2, 4, 1', 2', 6'	1a	6.04 s	102.5	CH <sub>2</sub>	1, 2
4		190.3	C		2		152.2	C	
4a		110.4	C		3	6.54 s	90.1	CH	1, 2, 5, 4, 6, 2'
5		144.2	C		4		155.9	C	
6		133.9	C		5		104.9	C	
6a	6.04 dd (0.8, 4.8)	102.9	CH <sub>2</sub>	6, 7	6		143.8	C	
7		155.2	C		1'a		155.8	C	
8	6.29 s	93.9	CH	4a, 5, 6, 7, 8a, 4	2'		147.5	C	
8a		161.4	C		3'		119.9	C	
1'		124.1	C		3'a		130.0	C	
2'		156.2	C		4'	7.44 d (7.6)	111.6	CH	1'a, 3'a, 5'
3'	6.89 d (7.6)	116.5	CH	1', 2', 5'	5'	7.27 m	122.9	CH	3'a
4'	7.10 m	129.3	CH	2', 3', 6'	6'	7.23 m	124.6	CH	1'a, 7'
5'	6.79 t (7.6)	120.5	CH	1', 2', 3', 6'	7'	7.80 d (7.3)	121.4	CH	1'a, 6'
6'	7.13 d (7.6)	130.9	CH	3, 2', 4'		3.72 s	57.0	OCH <sub>3</sub>	4
	3.92 s	60.8	OCH <sub>3</sub>			3.89 s	60.3	OCH <sub>3</sub>	6
	8.73 s		OH			4.58 s	56.4	CH <sub>2</sub> OH	2' 3', 3'a

<sup>a</sup> Spectra were recorded in acetone-d<sub>6</sub> at 400 MHz for <sup>1</sup>H NMR and 100 MHz for <sup>13</sup>C NMR.