Electronic Supplementary Information (ESI)

Deletion of a global regulator LaeB leads to the discovery of novel polyketides in *Aspergillus nidulans*

Haizhou Lin,[a,b,#] Haining Lyu[a,#], Shuang Zhou,[a,b] Jingwen Yu,[a,b] Nancy P. Keller,[c] Lin Chen,[a] and Wen-Bing Yin*[a,b]

[a] State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China.

[b] Savaid Medical School, University of Chinese Academy of Sciences, Beijing, 100049, China.

[c] Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, WI, 53706, USA

*Corresponding authors:

Wen-Bing Yin, E-mail: yinwb@im.ac.cn. Tel: 86-10-64806170.

Author Contributions

[#]These authors contributed equally.

Content

1. Supplementary methods

Strains, media and growth conditions

A. nidulans RJMP1.49 and its transformants were grown at 37°C on glucose minimum medium (GMM) for sporulation, transformation with appropriate nutrition as required.[1] *Escherichia coli* strain DH5α was propagated in LB medium with appropriate antibiotics for plasmid DNA.

Gene cloning, plasmid construction and genetic manipulations

Plasmids used in this study are listed in Table S2. The oligonucleotide sequences for PCR primers are given in Table S2. PCR amplification was carried out using the TransStart FastPfu DNA polymerase (Transgene Biotech) on a T100TM Thermal cycler from Bio-Rad. *A. nidulans* DNA was isolated using the previously described method.[2] Briefly, the marker gene cassette (*Aspergillus fumigatus pyrG*) was amplified by using pYH-WA (Table S1) as template and used as marker gene for *laeB* mutant. Approximately 1 kb fragments upstream and downstream of the target genes were amplified from genomic DNA of *A. nidulans* using the designated primers (Table S2), respectively. These three amplified PCR fragments were then purified with a Zymoclean Gel DNA Recovery Kit (Zymo research), quantified, and fused using fusion PCR procedures.[3] The final PCR product was amplified with the primer pairs nested primers or the end primers of each flanking region, confirmed fuses the three fragments into a single molecule and purified for fungal transformation. Fungal protoplast preparation and transformation were carried out as described by Bok & Keller.[4] Samples (5 μg) of the fusion PCR cassette were used to delete *laeB* by using *A. nidulans* strain RJMP1.49 (*pyrG89*, *pyroA4*, Δ*nkuA*::*argB*, *veA*) as the recipient host (Fig. S1 A). Disruption strains were verified by PCR (Fig. S1 B).

Chemical analysis and characterization of compounds

Analytical HPLC was conducted with a Waters HPLC system (Waters e2695, Waters 2998, Photodiode Array Detector) using an ODS column (C18, 250 × 4.6 mm, YMC Pak, 5 μm) with a flow rate of 1 mL/min. Fresh extracts were dissolved in methanol (5 mg/mL) before separated on a linear gradient of methanol:H_2O (0.1% formic acid) at a flow rate of 1 mL/min. Fresh extracts from Δ*laeB* mutants were detected for 30 min using a linear gradient of 20% to 100% (0–20 min), 100% methanol (20–25 min), and 20% methanol (25–30 min). The *laeB* mutant was cultivated on 2 kg rice at 37°C for 30 days. Then, the fermentation was extracted three times with ethyl acetate. The organic solvent was evaporated to dryness under vacuum to obtain 33.5 g of crude extract. The extract was fractionated on a silica gel chromatographic column (CC) using petroleum ether, dichloromethane, ethyl acetate, and methanol to give 4 fractions (Fractions 1–4, the HPLC of Fraction 2 was showed as Fig. 2 in the article). The subfraction 2 (8.8 g) was further purified by silica gel CC eluted with petroleum ether/ethyl acetate (16:1, 8:1, 4:1, and 0:1) to give seven subfractions (Fr. 2-1 to Fr. 2-7). The Fr. 2-6 was isolated by semi-preparative HPLC with CH_3CN/H_2O (0.1% formic acid) (55:45, 2.5mL/min) to afford **3** (1.8 mg, t_R 12.1 min), **2** (1.8 mg, t_R 13.4 min), **6** (1.5 mg, t_R 16.2 min), **5** (5.1 mg, t_R 15.4 min), and **8** (12.1 mg, t_R 17.3 min, write solid). The subfraction 3 (6.0 g) was further purified by silica gel CC eluted with petroleum ether/acetone (20:1, 10:1, 4:1, and 0:1) to give seven subfrations (Fr. 3-1 to Fr. 3-7). The subfraction 3-7 was purified by silica gel CC followed CC on Sephadex LH-20 (50:50 CH_2Cl_2/CH_3OH) to afford eight fractions. The Fr. 3-7-1 was purified by semi-preparative HPLC (30:70 CH_3CN/H_2O, 0.1% formic acid, 2.5mL/min) to afford **1** (3.0 mg, t_R 12.1 min yellow solid), and the Fr. 3-7-2 was separated by thin layer chromatography (TLC) eluted with

dichloromethane/methanol (25:1) to afford **4** (14.0 mg, t_R 10.4 min, white solid). Fr. 3-7-6 was isolated by semi-preparative HPLC (45:55 CH_3CN/H_2O, 0.1% formic acid, 2.5mL/min) to afford **7** (4.8 mg, t_R 15.7 min, light red solid).

Compound **2**: yellow solid (MeOH); $[\alpha]^{24}_D$ +2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.07), 259 (3.52), 300 (3.20) nm; IR (KBr) ν_{max} 2918, 1742, 1626, 1463, 1427, 1327, 1260, 1237, 1126, 1202, 1113, 971, 903 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m*/*z* 225.07587 $[M + H]^+$ (calcd for $C_{11}H_{12}O_5$, 225.07575).

Compound **3**: yellow solid (MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.11), 254 (3.74), 314 (3.42) nm; IR (KBr) ν_{max} 3444, 2961, 2916, 2850, 1748, 1575, 1464, 1261, 1046, 1037, 1028, 957, 859, 801, 705 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m*/*z* 225.07558 $[M + H]^+$ (calcd for $C_{11}H_{12}O_5$, 225.07575).

Compound **5**: brown solid (MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.89), 235 (4.15), 284 (3.17) nm; IR (KBr) ν_{max} 3446, 2953, 2927, 1592, 1520, 1504, 1475, 1390, 1350, 1322, 1265, 1224,1178, 1107, 1086, 996, 930, 821, 712 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m*/*z* 275.09195 $[M + H]^+$ (calcd for $C_{15}H_{14}O_5$, 275.09140).

Compound **6**: brown solid (MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.06), 236 (4.20), 282 (3.28) nm; IR (KBr) ν_{max} 3340, 2931, 1595, 1515, 1475, 1358, 1223, 1140, 1120, 1090, 1060, 1027, 830 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m*/*z* 275.09182 $[M + H]^+$ (calcd for $C_{15}H_{14}O_5$, 275.09140).

Analytical methods and equipment overview

NMR spectra (^1H, ^{13}C, HSQC, HMBC) were recorded on a Bruker Avance-500 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. HR-ESI-MS utilized on an Agilent Accurate-Mass-QTOF LC/MS 6520 instrument. HPLC analysis was performed on a Waters HPLC system (Waters e2695, Waters 2998, Photodiode Array Detector) using an ODS column (C18, 250 × 4.6 mm, YMC Pak, 5 μm) with a flow rate of 1 mL/min. Semi-preparative HPLC was performed using an ODS column [HPLC (YMC-Pack ODS-A, 10 × 250 mm, 5 μm, 3 mL/min)]. Column chromatography (CC) were performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), and Sephadex LH-20 (Amersham Biosciences), respectively.

2. Supplementary tables

Table S1 Fungal strains and plasmids used in this study.

Strain/plasmid	Description	Reference
A. nidulans RJMP1.49	*pyrG89*, *pyroA4*, Δ*nkuA*::*argB*, *veA*	5
TYJW2.3	*laeB* mutant	This study
TYZS13.1	p*YH-WA* in RJMP1.49 (Isogenic control)	This study
DH5α	*Escherichia coli* strain	
p*YH-WA*	Gain maker *pyrG*	This study

Table S2 PCR primers sets for the disruption of *laeB* in this study.

Name	Oligonucleotide sequence (5'-3')	Uses
KO AN4699 5F-F	CATGCGGACTGAGGAGTTCAATC	5' flanking region amplification
KO AN4699 5F-R	CATATTTCGTCAGACACAGAATAA CTGCAGCGACTGGCTATATAC	5' flanking region amplification
KO AN4699 3F-F	GCATCAGTGCCTCCTCTCAGACAGA ATGGACCTAGAACAGAATTAGAGGC	3' flanking region amplification
KO AN4699 3F-R	CAGTCATGACTGGCGTTGTC	3' flanking region amplification
UU maker F	GAGAGTTATTCTGTGTCTGACG	Maker *pyrG* amplification
UU maker R	ATTCTGTCTGAGAGGAGGC	Maker *pyrG* amplification
FP KO AN4699 F	CTGTTGATACTCTTGGTGGAGGC	Fusion PCR for three cassette segments
FP KO AN4699 R	GCCTGGTGATAATCCGGTAAGG	Fusion PCR for three cassette segments
RT AN4699 F	CTTGCCTTCAGGCAGTGTG	Diagnostic PCR for *laeB* deletion
RT AN4699 R	CATCACCATCACGCTCAACC	Diagnostic PCR for *laeB* deletion
Detect KO AN4699 5F-F	GGCTAGGATAGAGCTGAAGAGAAG	Diagnostic PCR for *laeB* deletion
Detect KO AN4699 MAKER R	CTGTAGTCAGGTACAGCTAGAATGG	Diagnostic PCR for *laeB* deletion
Detect KO AN4699 MAKER F	GCTTATATGGCCAGAGTATGCG	Diagnostic PCR for *laeB* deletion
Detect KO AN4699 3F-R	GAGTTGACGAGCAACGAGTC	Diagnostic PCR for *laeB* deletion

3. Supplementary figures

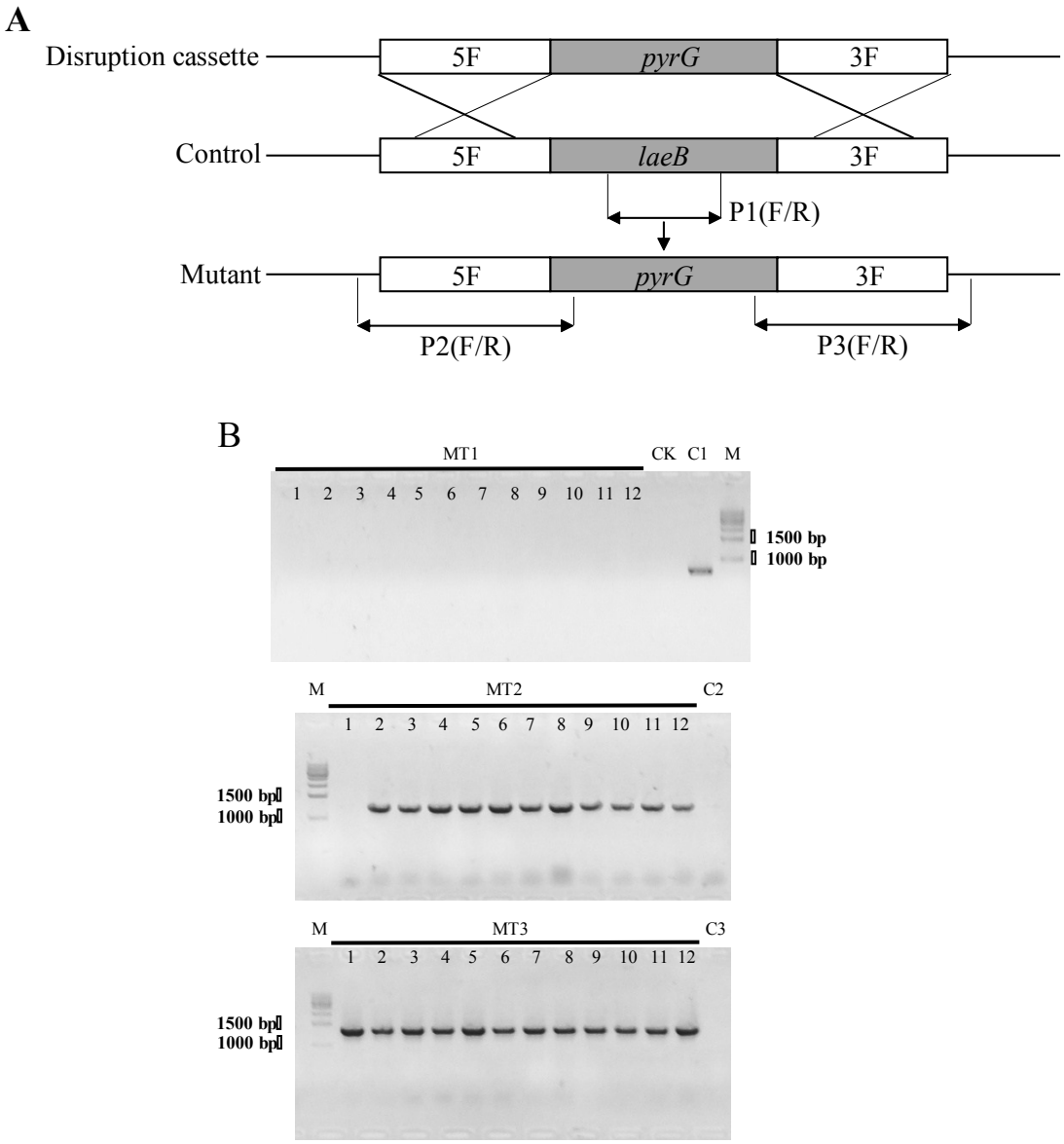

Fig. S1 Generation of *laeB* disruption strains. A. Schematic illustration for disruption of targeted genes. The *pyrG* gene in vector pYH-WA was used as a selection maker after transformation. Transformation was performed by homologous recombination via protoplast transformation methods. Three pairs of primers including P1F/R, P2F/R and P3F/R were used for transformant screening. B. Confirmation of *laeB* disruption strains by diagnostic PCR, specific bands (ca. 1.0-1.5 kb) were only found in mutants but not in control.

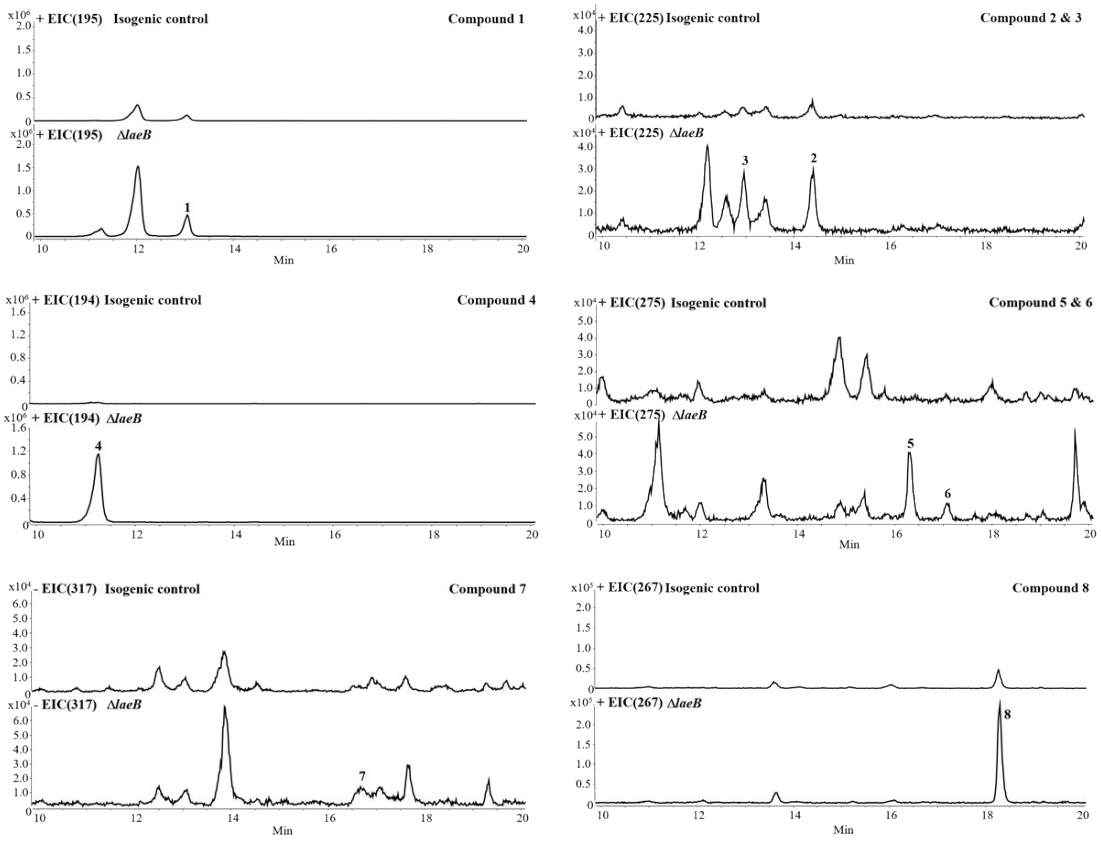

Fig. S2 The extracted ion chromatography (EIC) of compounds **1**-**8** from *A. nidulans* isogenic control strain and Δ*laeB* mutant. The production of compounds **1**, **2**, **3**, **7**, and **8** were increased in Δ*laeB* mutant compared with the isogenic control, and compounds **4**, **5**, **6** were newly produced in the Δ*laeB* mutant. The LC-MS results coupled with HPLC data and literature information[6] revealed that the deletion of LaeB resulted in the low-expression of some known SMs[6], on the other hand, the production of some low-expressed or silent SMs such as **5** and **6** in the original strain raise up.

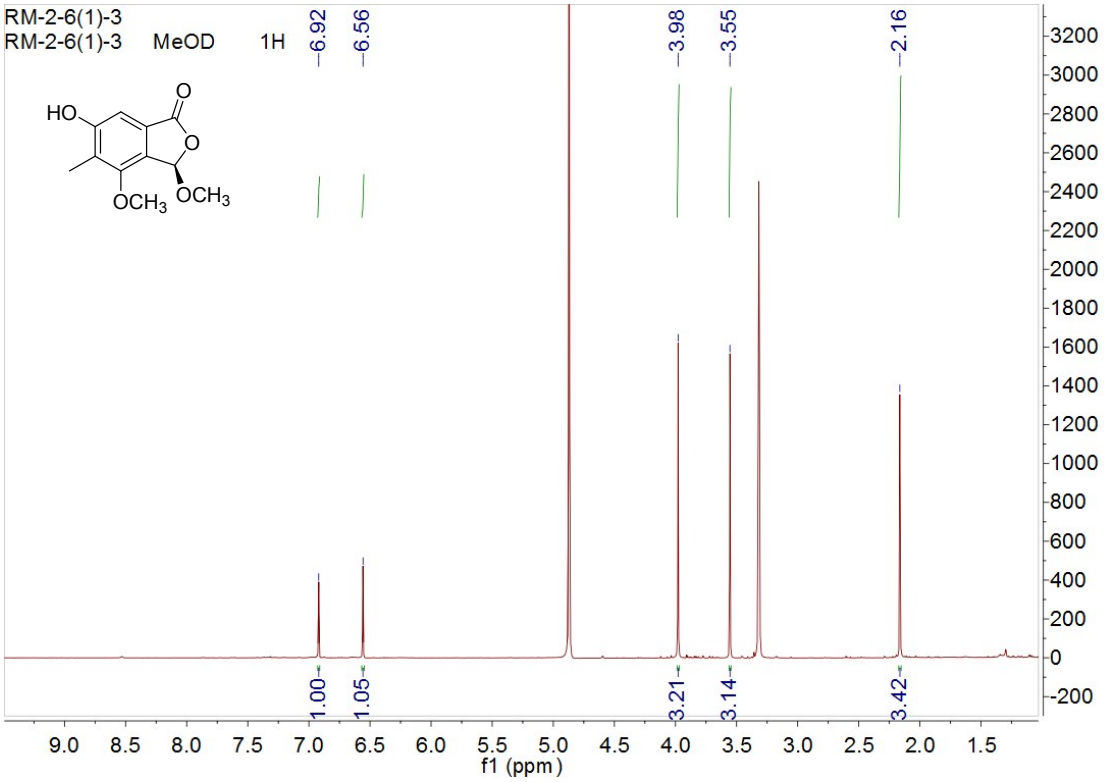

Fig. S3 ^{1}H NMR spectrum of **2** in methanol-d_4.

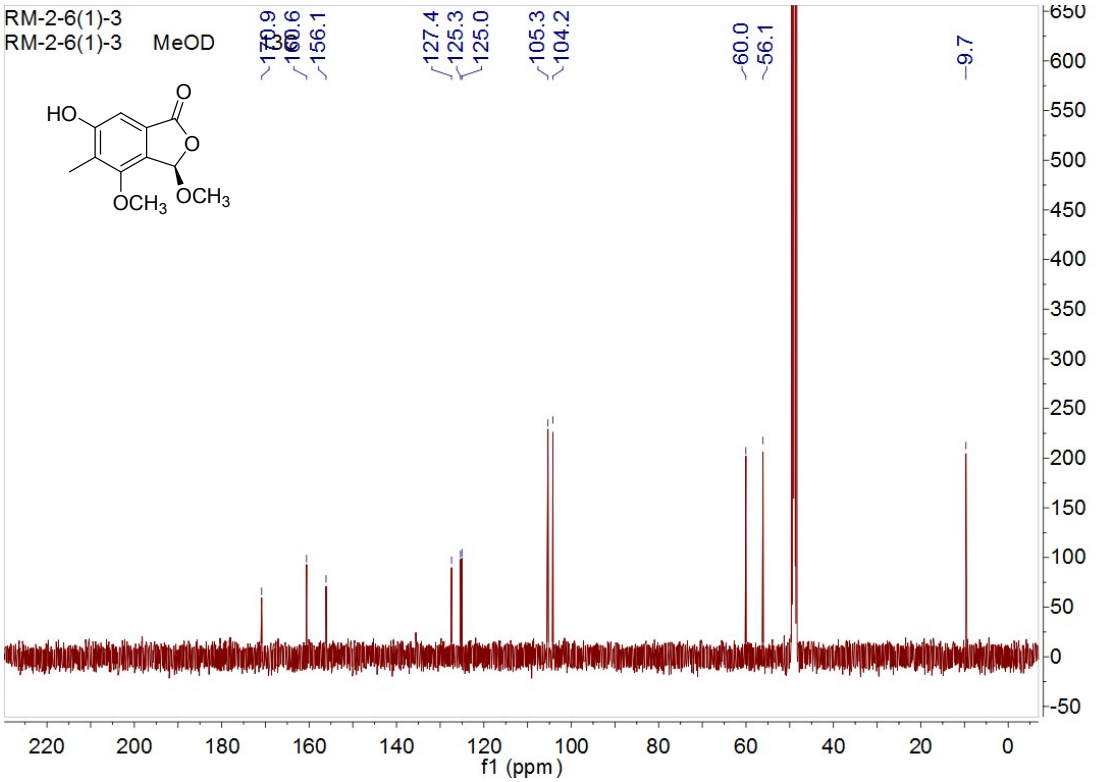

Fig. S4 ^{13}C NMR spectrum of **2** in methanol-d_4.

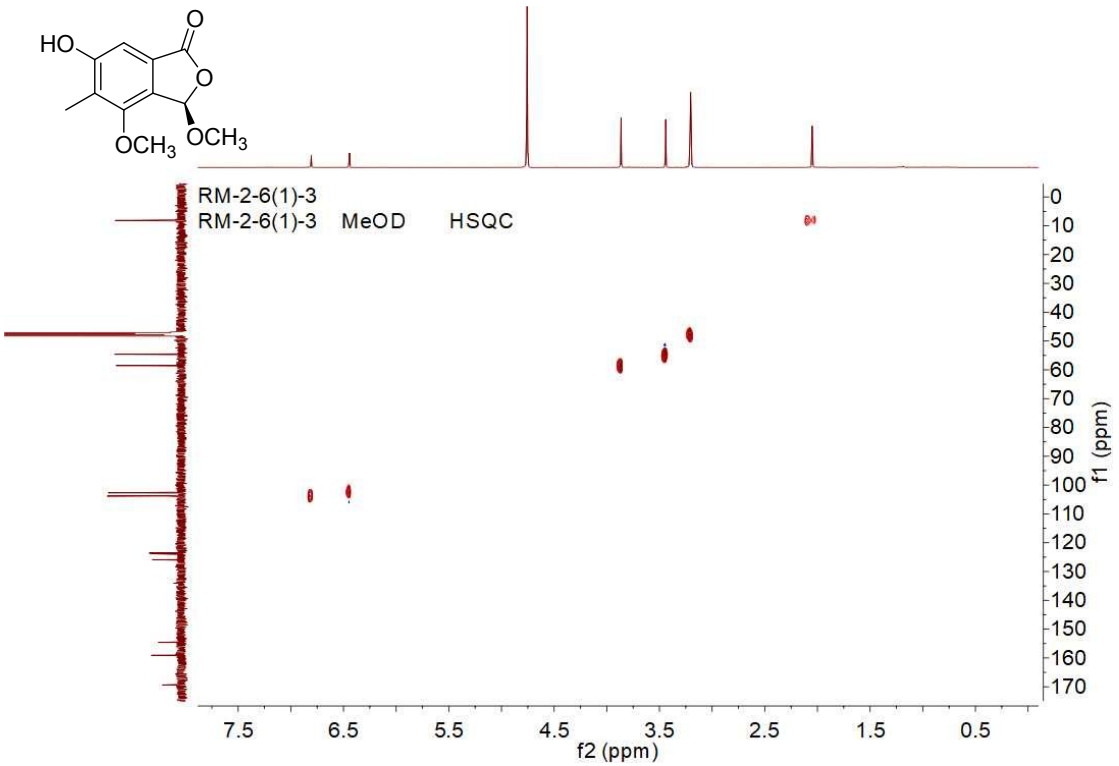

Fig. S5 HSQC spectrum of **2** in methanol-d_4.

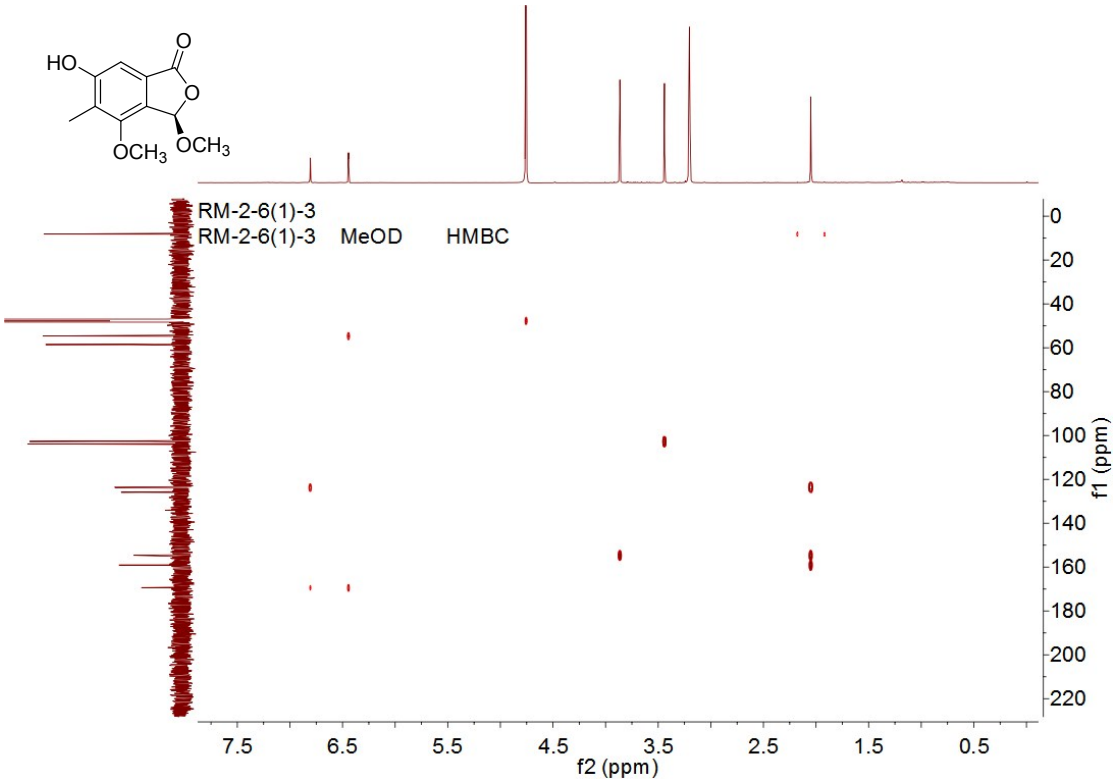

Fig. S6 HMBC spectrum of **2** in methanol-d_4.

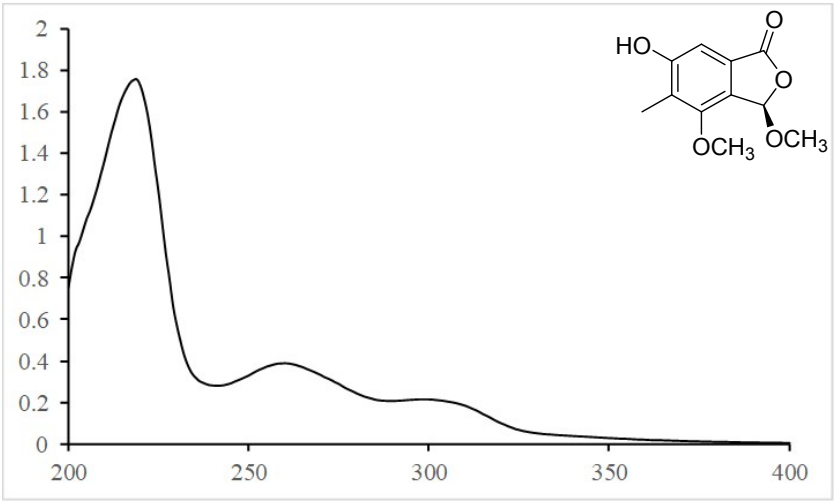

Fig. S7 UV sperctrum of **2**.

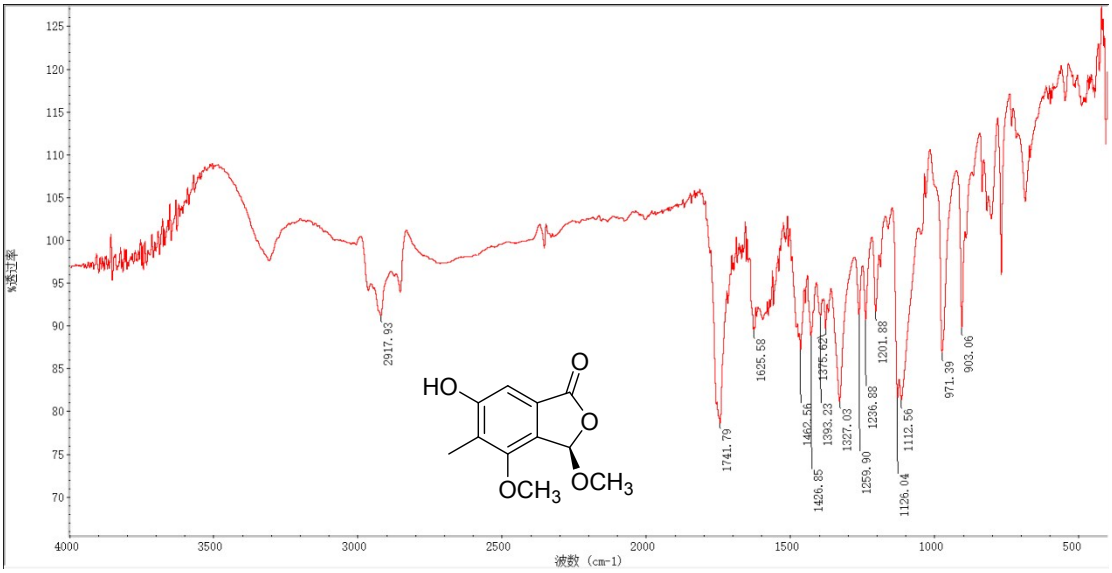

Fig. S8 IR sperctrum of **2**.

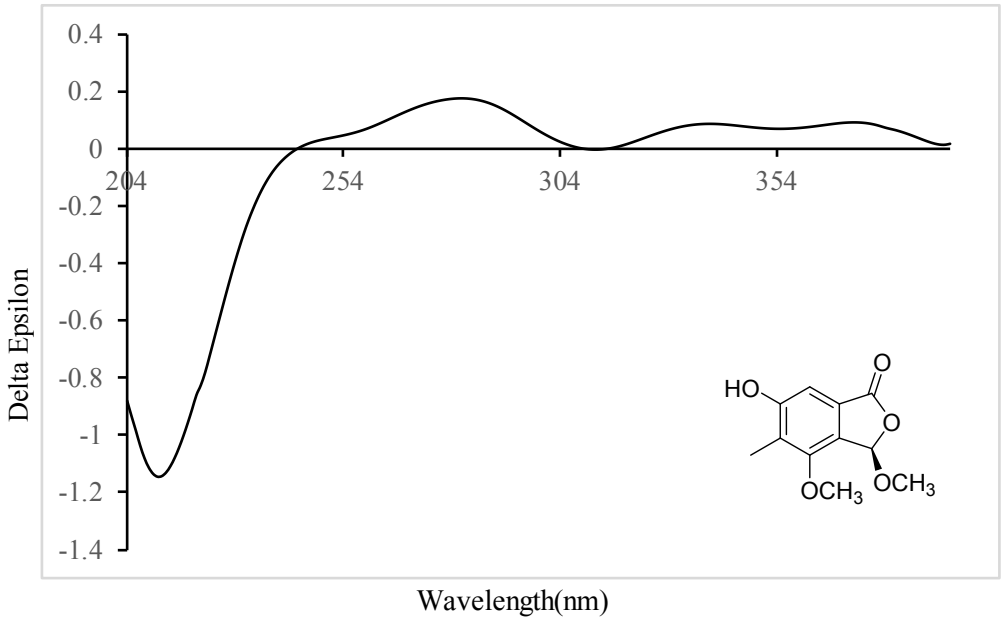

Fig. S9 ECD sperctrum of **2**.

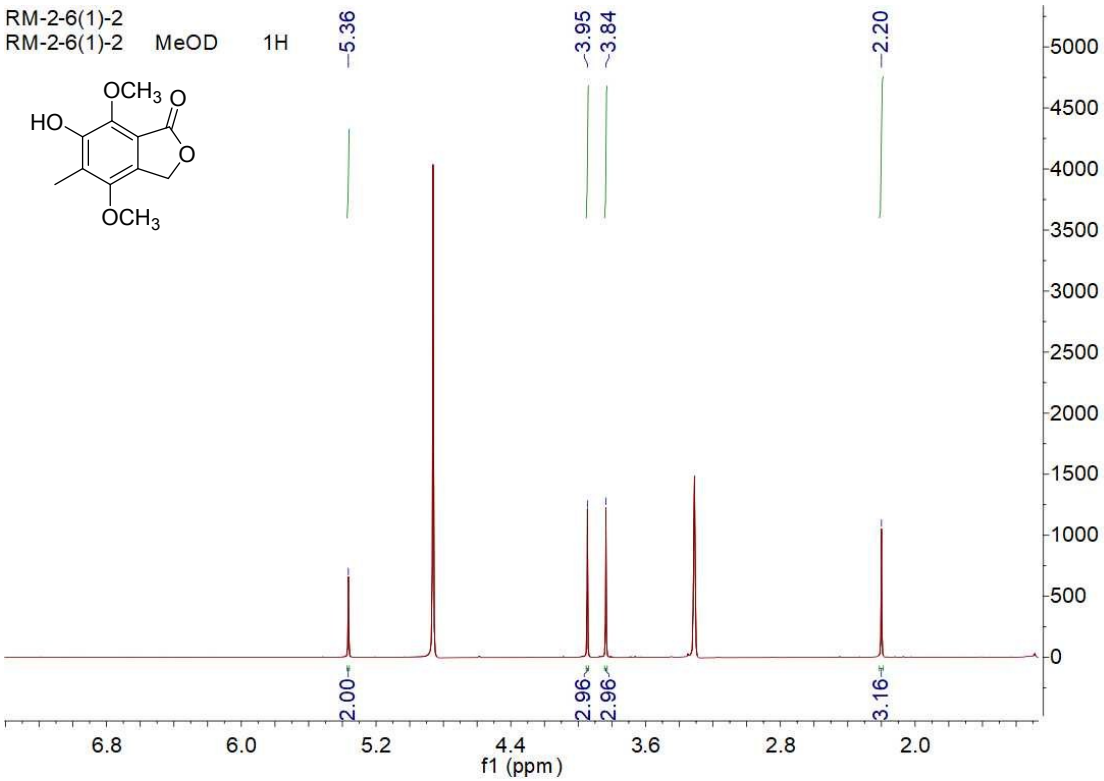

Fig. S10 ^{1}H NMR spectrum of **3** in methanol-d_4.

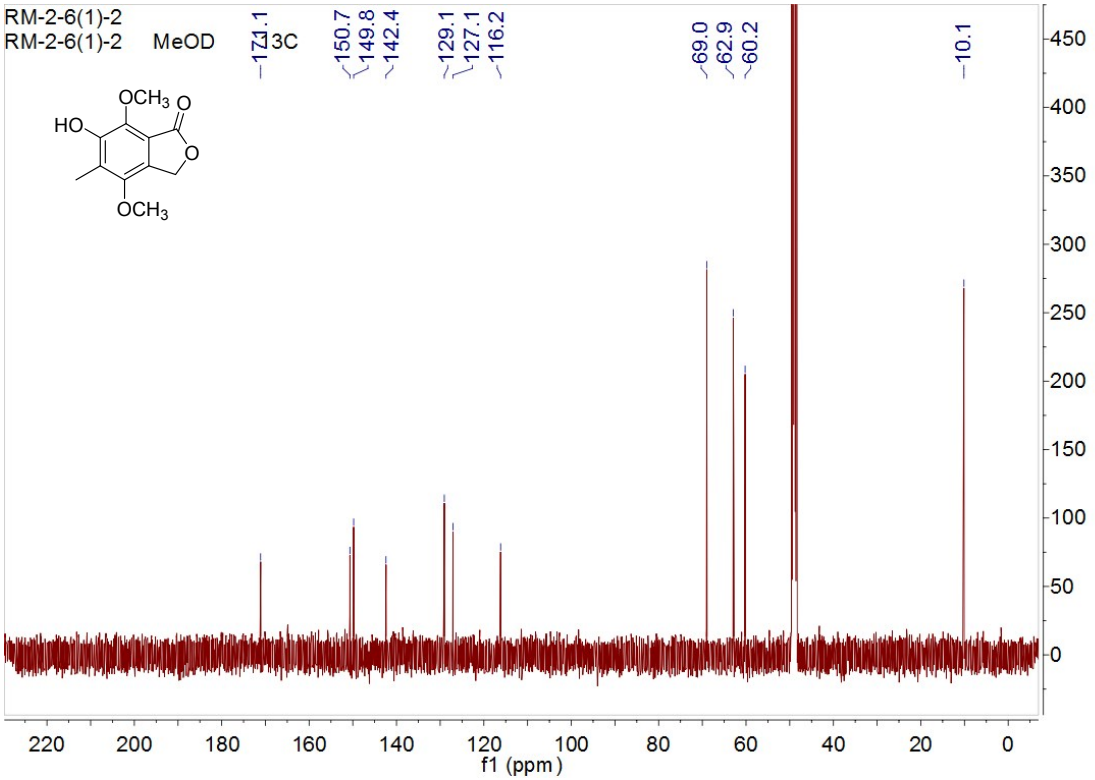

Fig. S11 ^{13}C NMR spectrum of **3** in methanol-d_4.

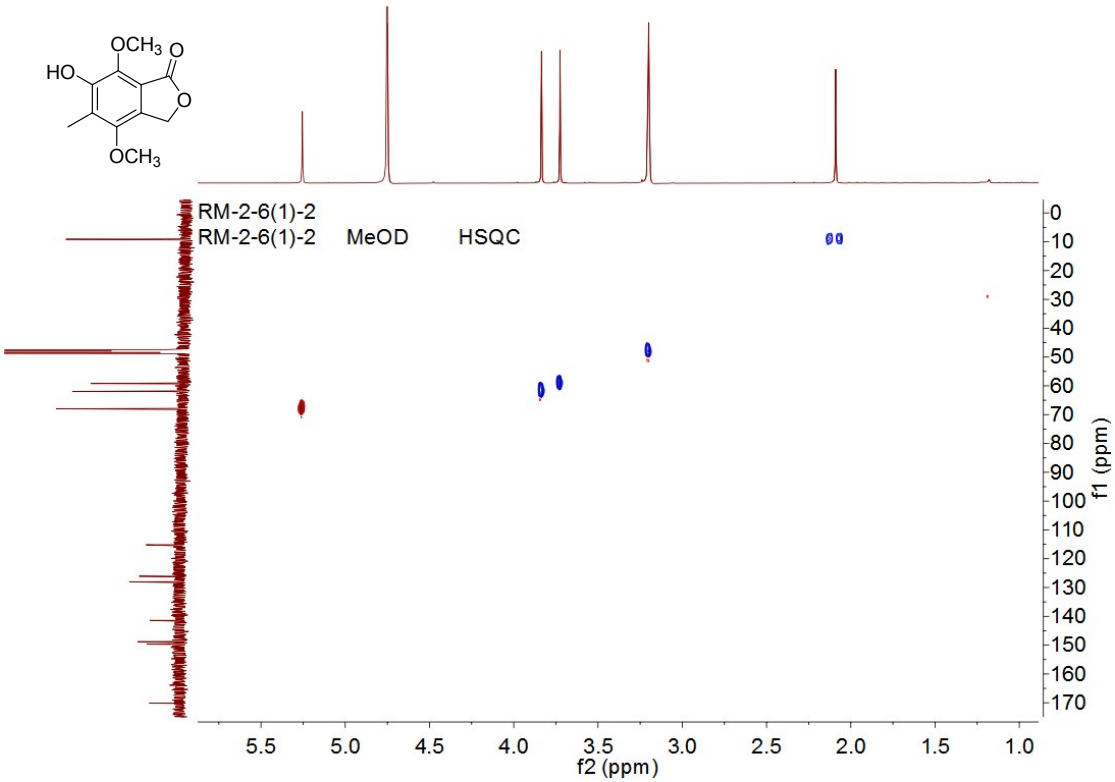

Fig. S12 HSQC spectrum of **3** in methanol-d_4.

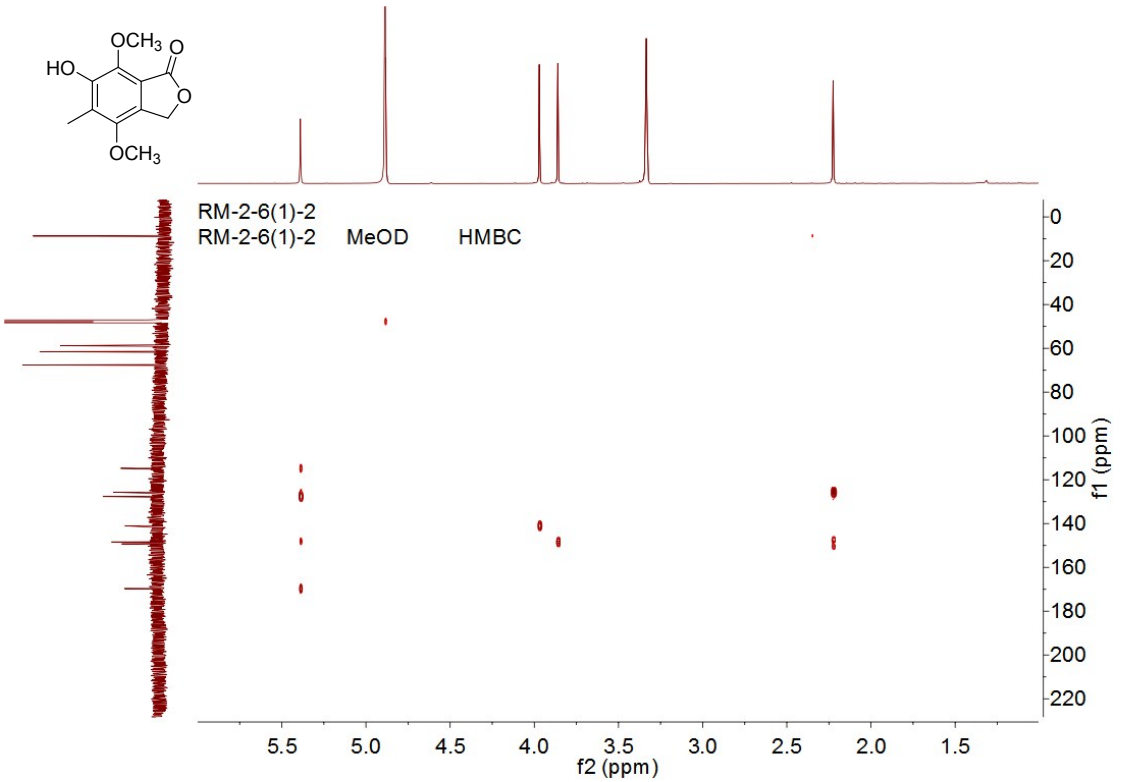

Fig. S13 HMBC spectrum of **3** in methanol-d_4.

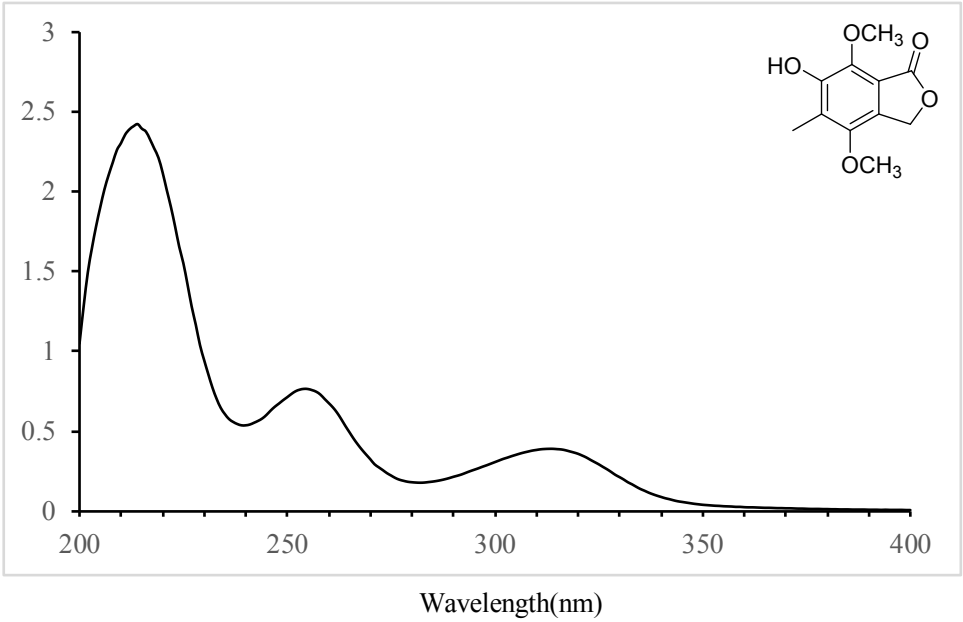

Fig. S14 UV sperctrum of **3**.

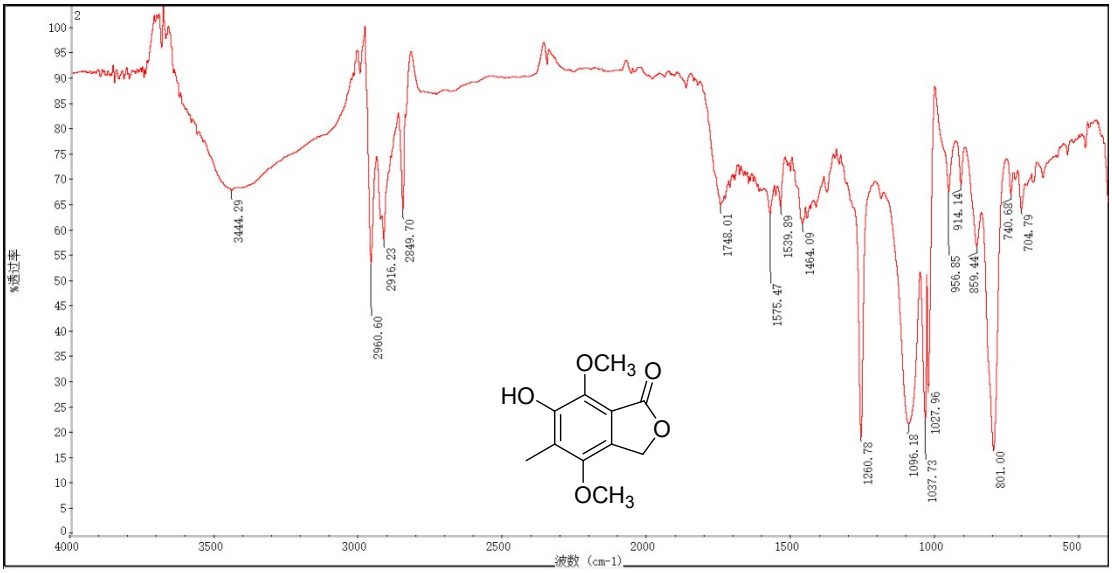

Fig. S15 IR sperctrum of **3**.

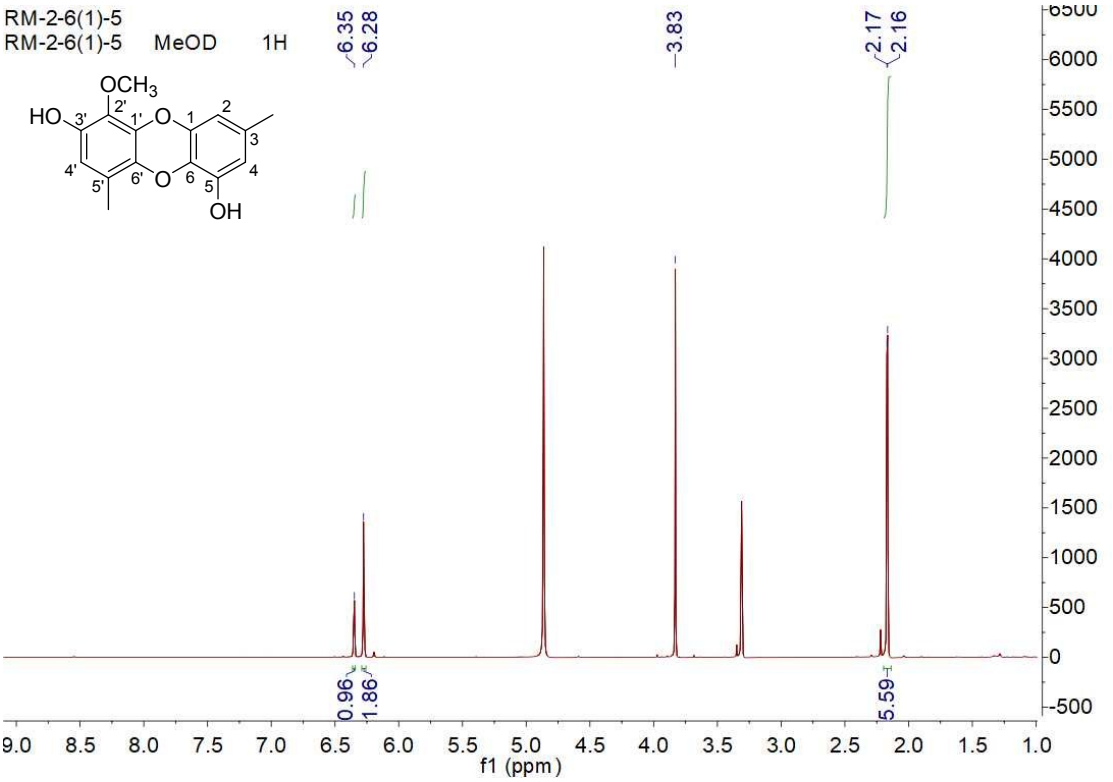

Fig. S16 ^{1}H NMR spectrum of **5** in methanol-d_4.

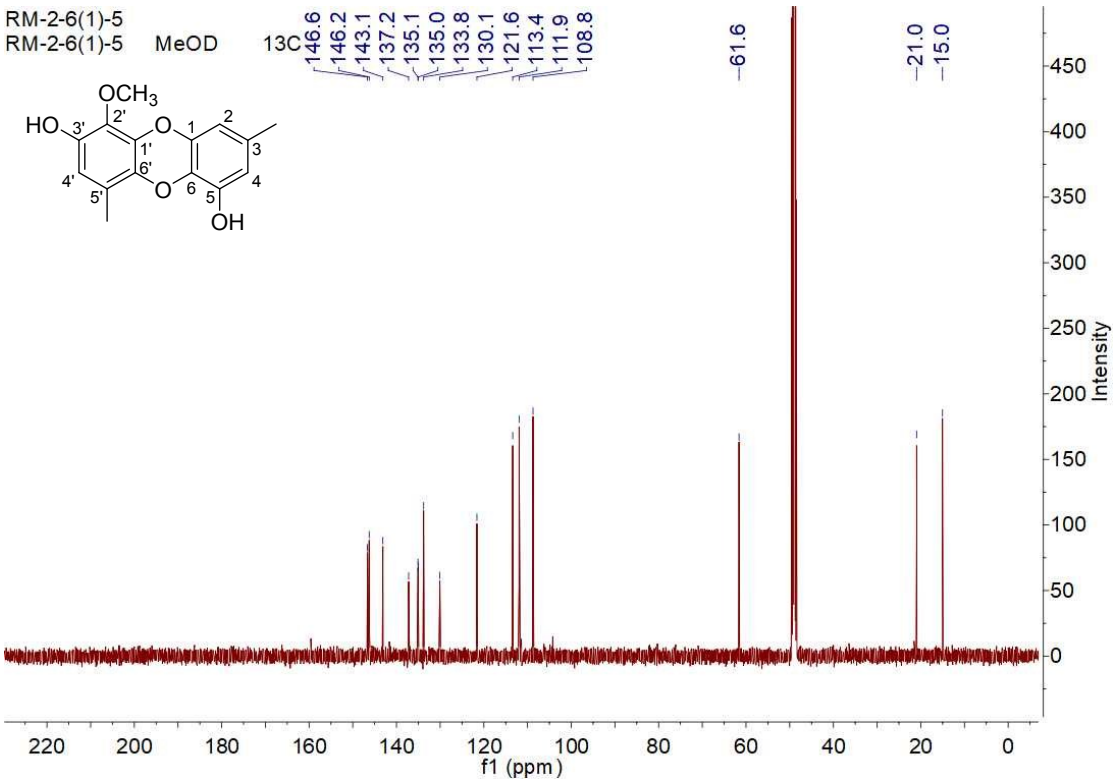

Fig. S17 ^{13}C NMR spectrum of **5** in methanol-d_4.

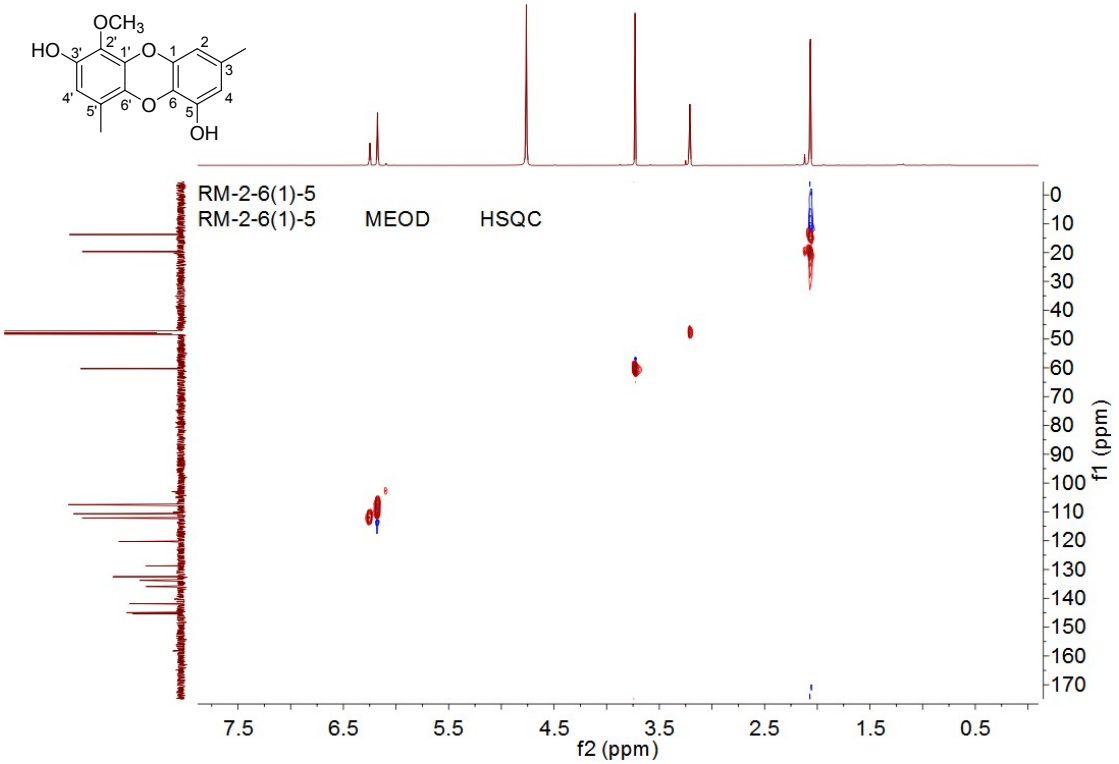

Fig. S18 HSQC spectrum of **5** in methanol-d_4.

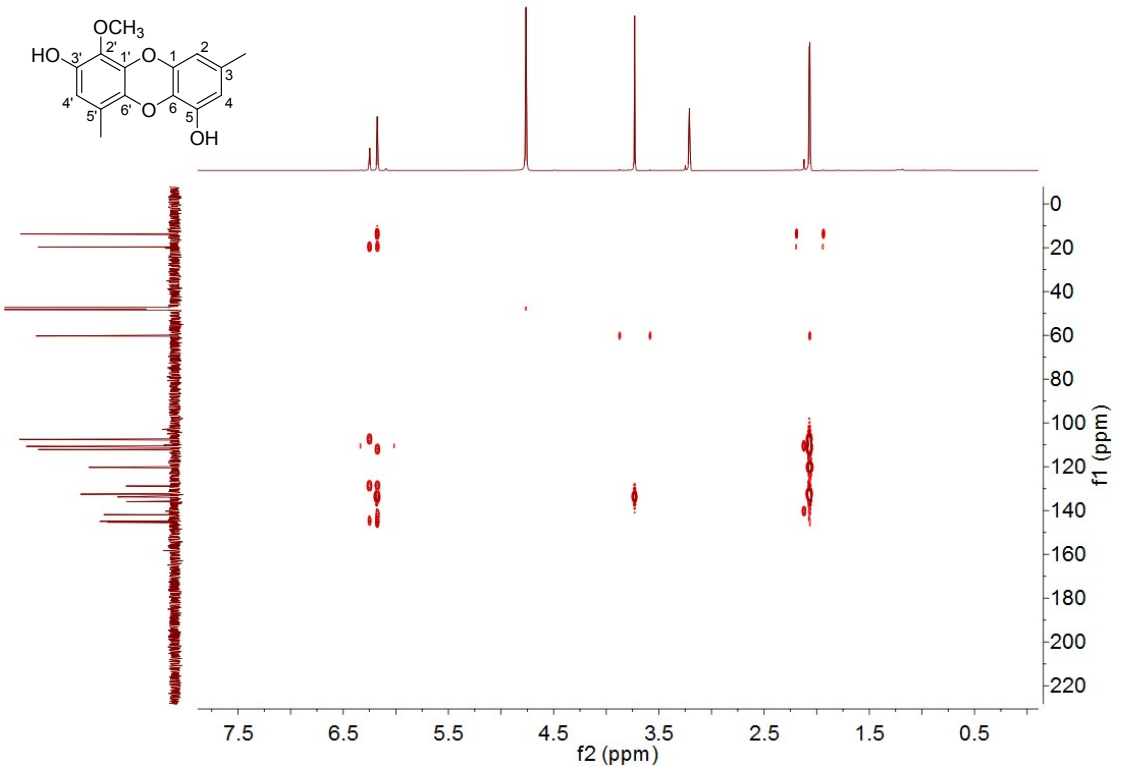

Fig. S19 HMBC spectrum of **5** in methanol-d_4.

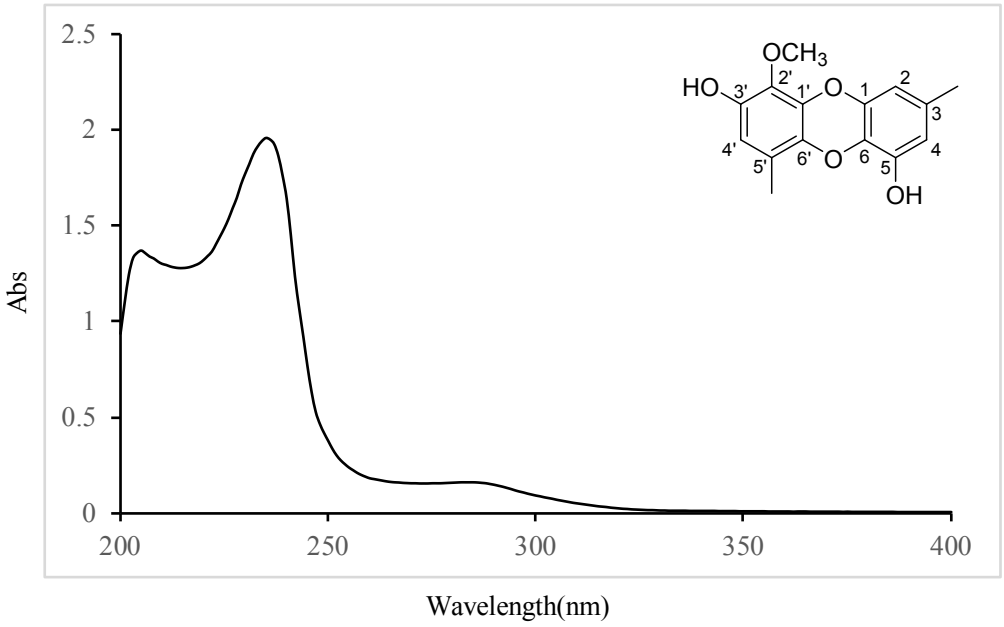

Fig. S20 UV spectrum of **5**.

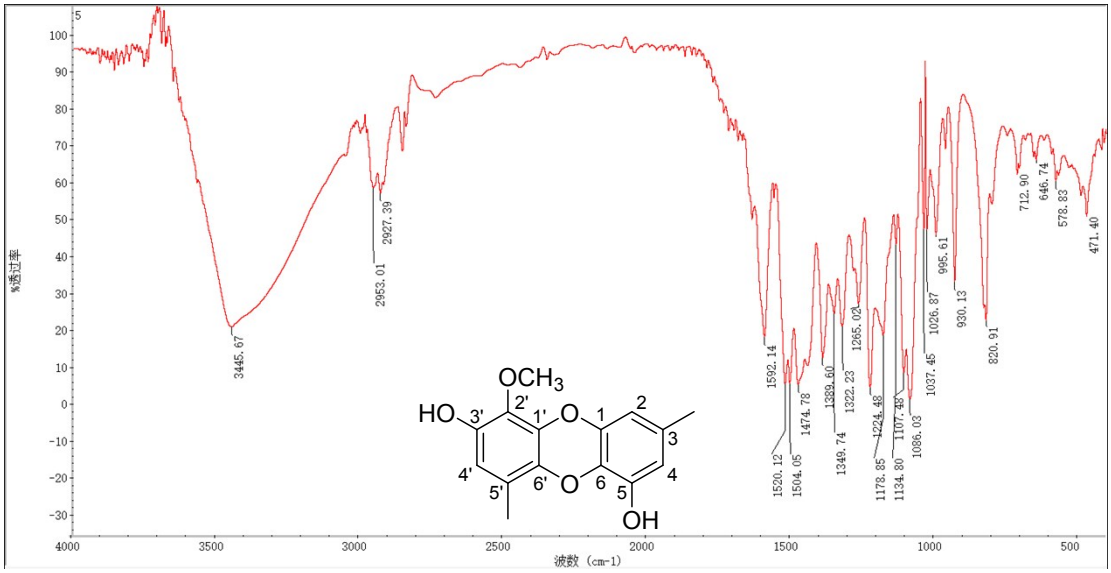

Fig. S21 IR spectrum of **5**.

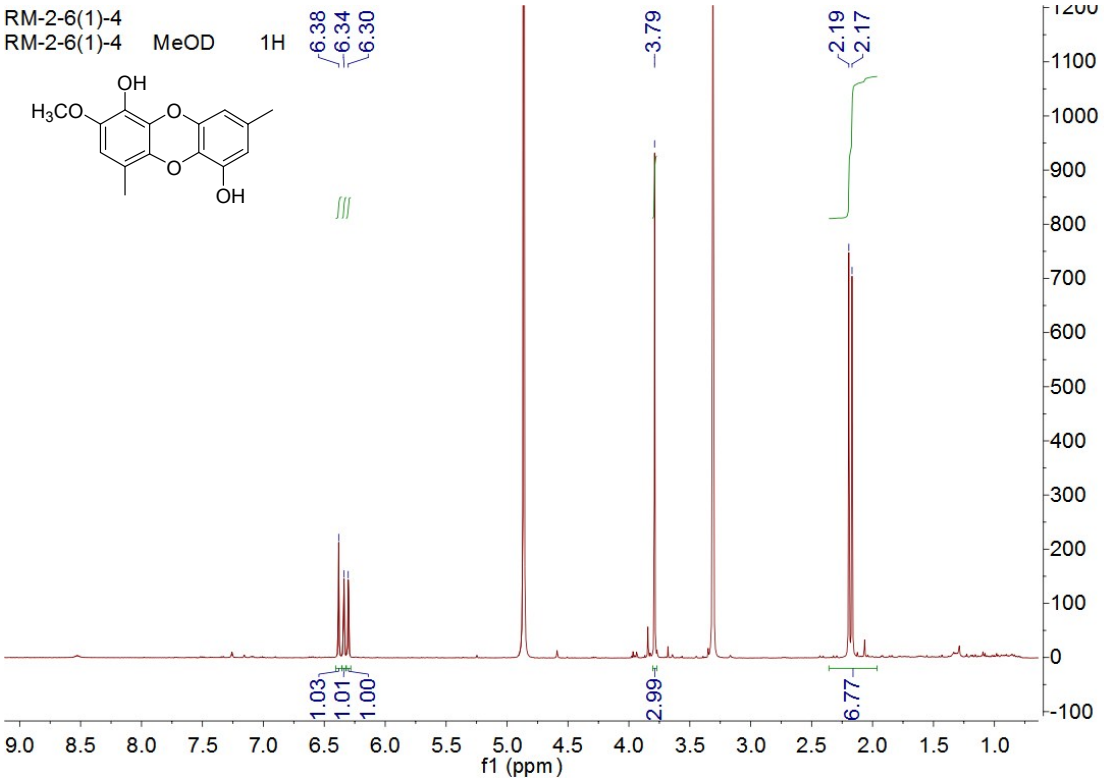

Fig. S22 ^{1}H NMR spectrum of **6** in methanol-d_4.

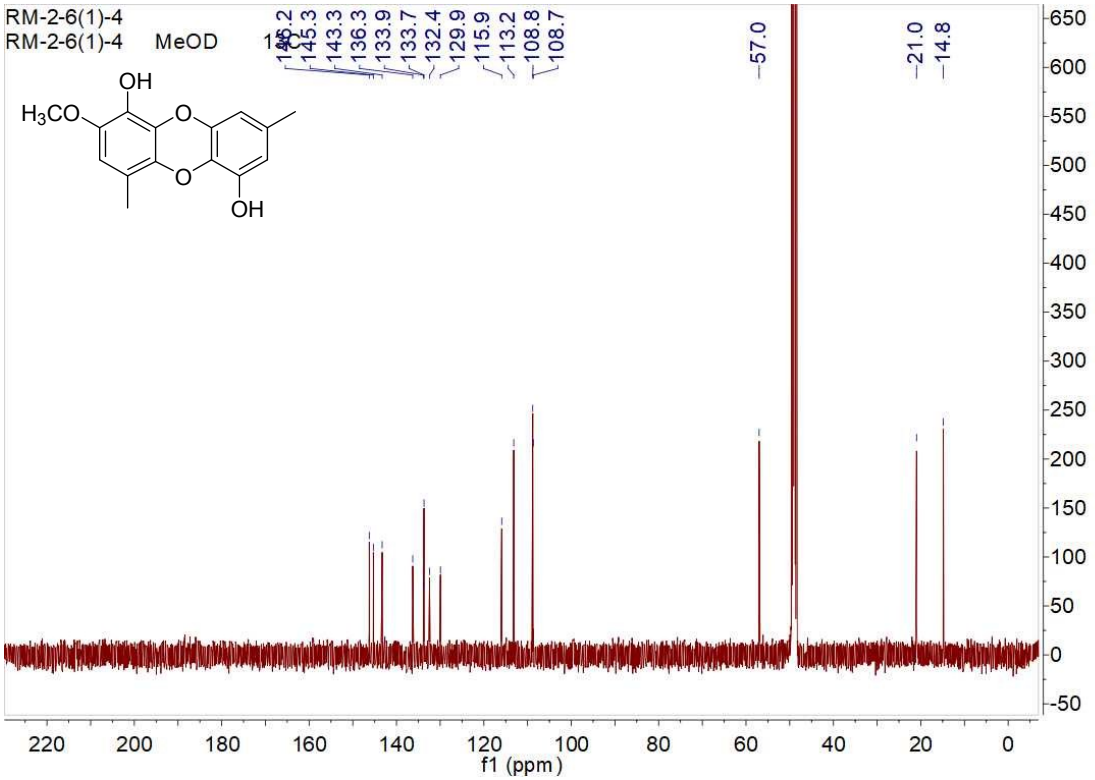

Fig. S23 ^{13}C NMR spectrum of **6** in methanol-d_4.

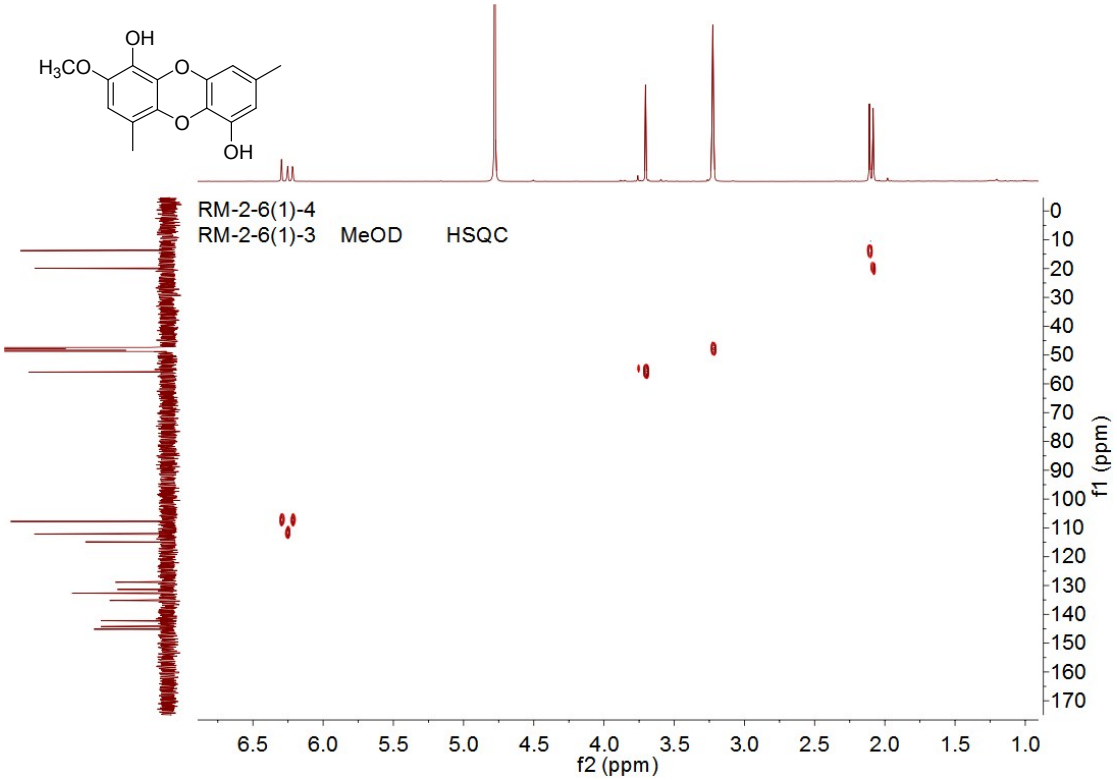

Fig. S24 HSQC spectrum of **6** in methanol-d_4.

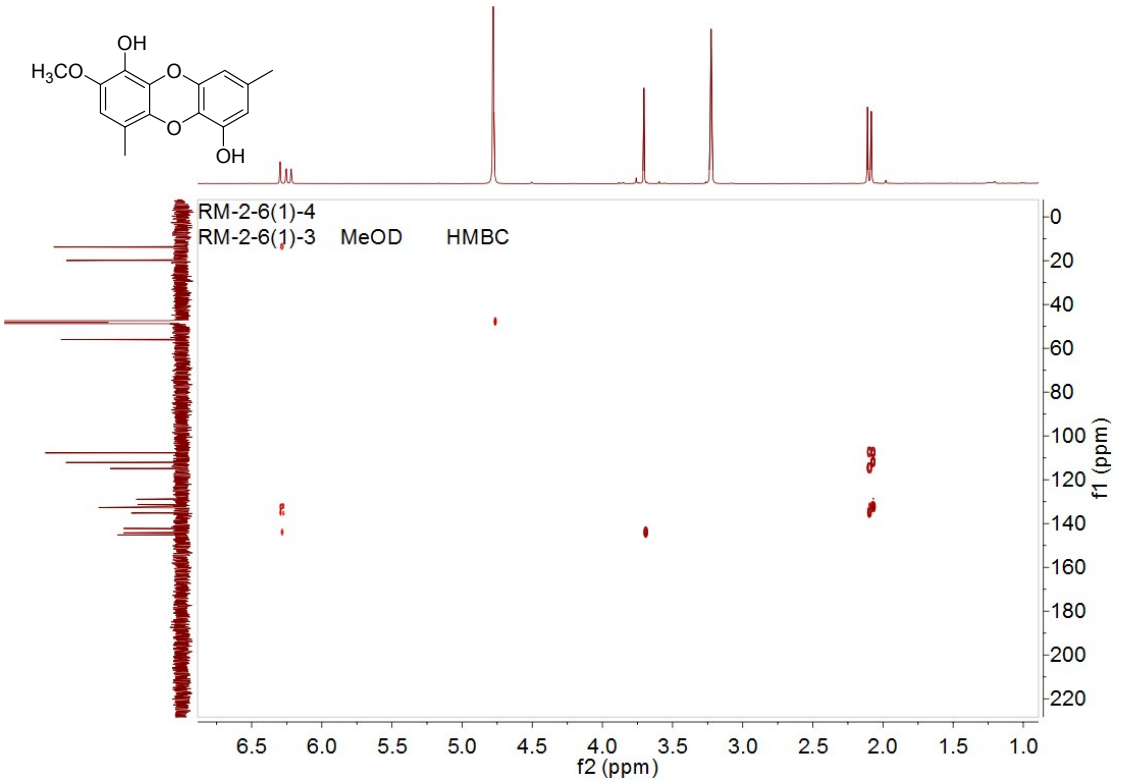

Fig. S25 HMBC spectrum of **6** in methanol-d_4.

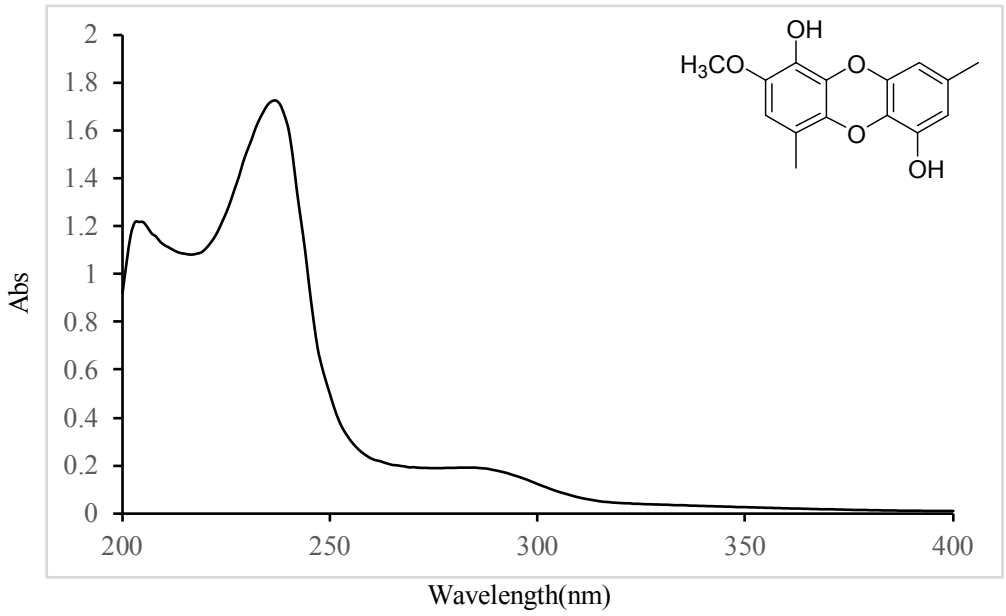

Fig. S26 UV spectrum of **6**.

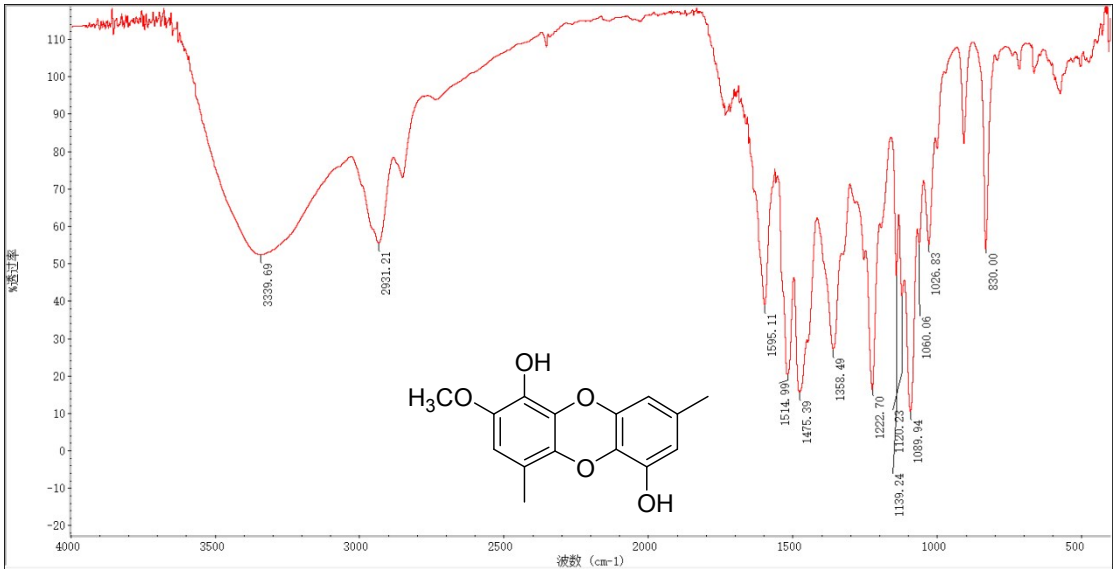

Fig. S27 IR spectrum of **6**.

4. Supplementary references

1. K. Shimizu and N. P. Keller, *Genetics*, 2001, **157**, 591-600.
2. T. Asai, S. Morita, T. Taniguchi, K. Monde and Y. Oshima, *Organic & Biomolecular Chemistry*, 2016, **14**, 646-651.
3. E. Szewczyk, T. Nayak, C. E. Oakley, H. Edgerton, Y. Xiong, N. Taheri-Talesh, S. A. Osmani and B. R. Oakley, *Nat Protoc*, 2006, **1**, 3111-3120.
4. J. W. Bok and N. P. Keller, *Eukaryot Cell*, 2004, **3**, 527-535.
5. M. I. Shaaban, J. W. Bok, C. Lauer and N. P. Keller, *Eukaryot Cell*, 2010, **9**, 1816-1824.
6. B. T. Pfannenstiel, X. X. Zhao, J. Wortman, P. Wiemann, K. Throckmorton, J. E. Spraker, A. A. Soukup, X. Y. Luo, D. L. Lindner, F. Y. Lim, B. P. Knox, B. Haas, G. J. Fischer, T. Choera, R. A. E. Butchko, J. W. Bok, K. J. Affeldt, N. P. Keller and J. M. Palmer, *Mbio*, 2017, **8**(5):e01246-17.