Supporting Information

Lasiodiplactone A, a Novel Lactone from the Mangrove Endophytic Fungus *Lasiodiplodia theobromae* ZJ-HQ1

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Fig. S1 HREIMS spectrum of **1**

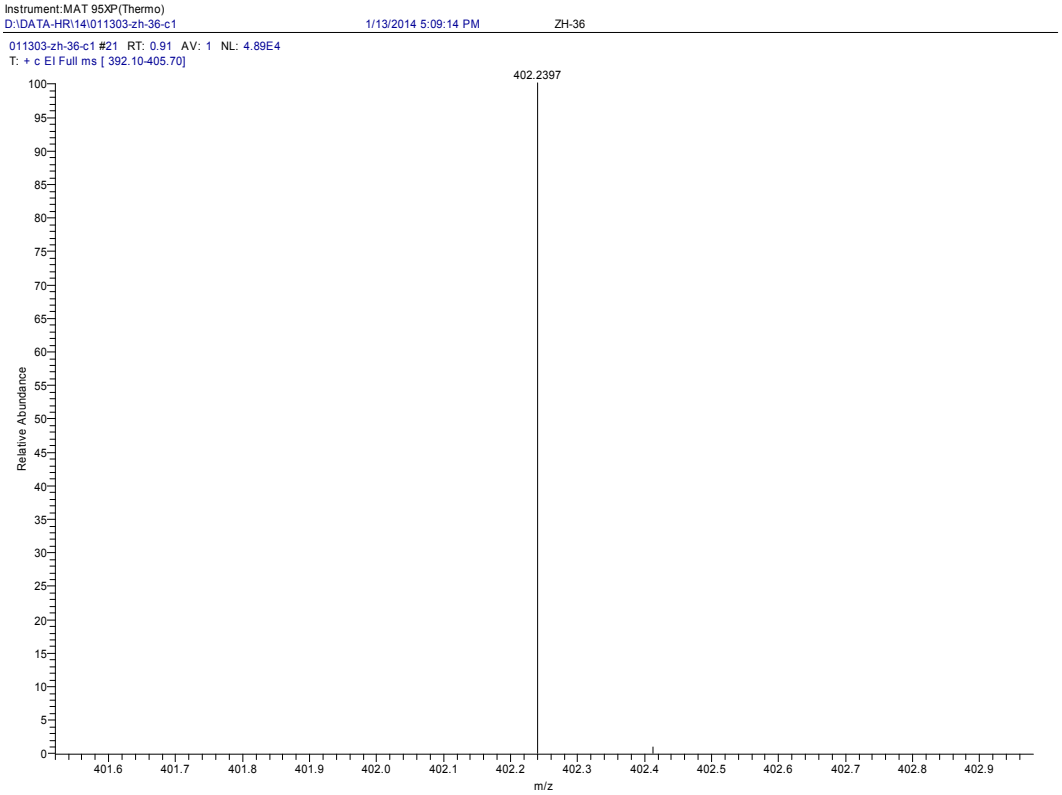


Fig. S2 ^{1}H NMR spectrum of **1** in $CDCl_3$

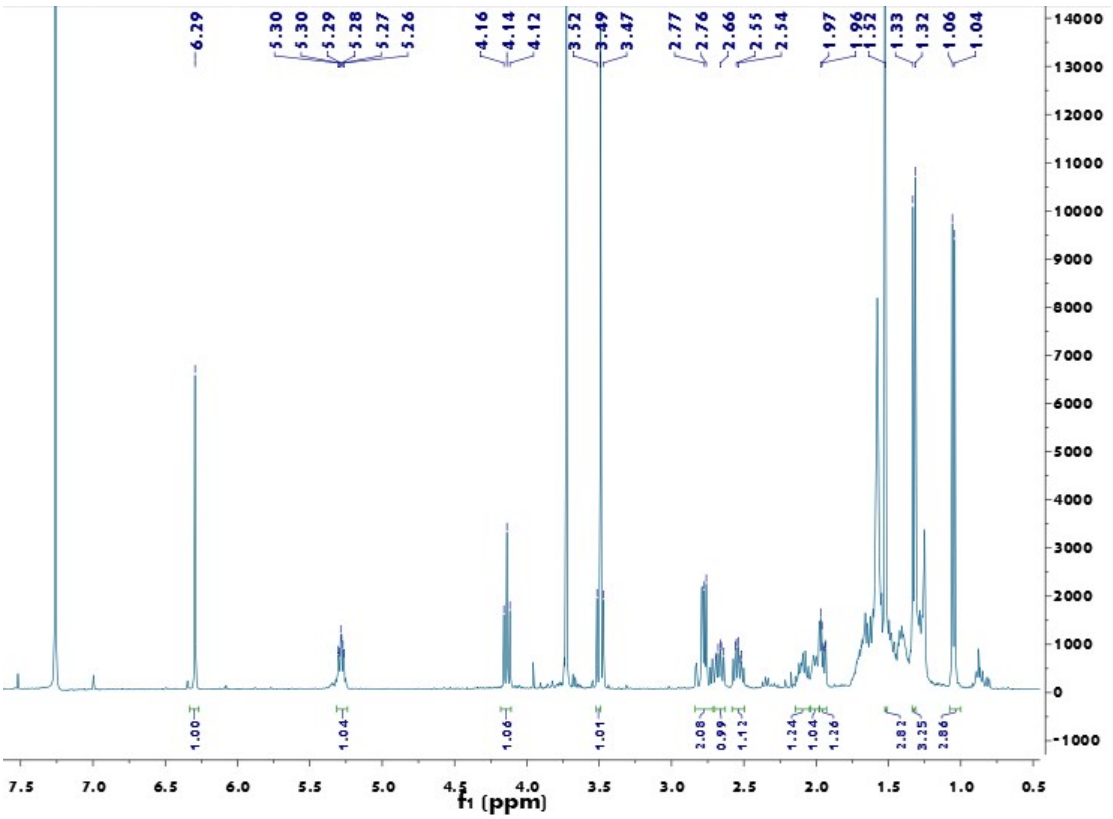


Fig. S3 ^{13}C NMR spectrum of **1** in $CDCl_3$

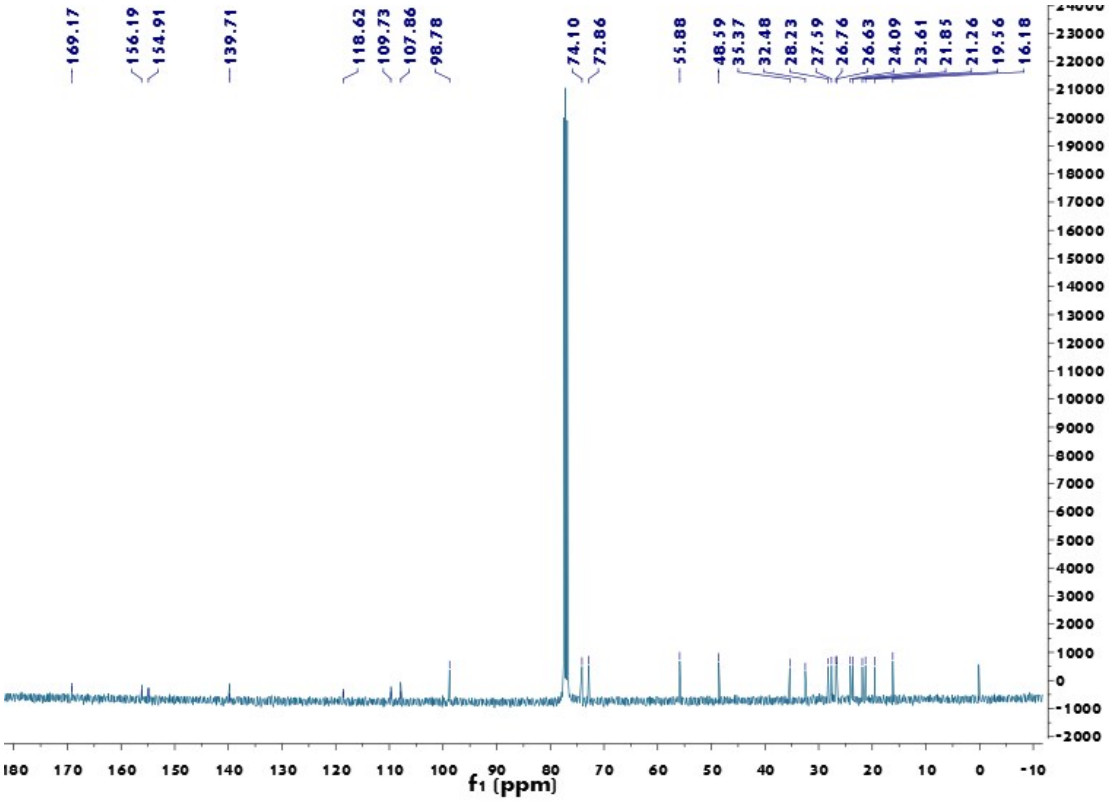


Fig. S4 HSQC spectrum of **1** in $CDCl_3$

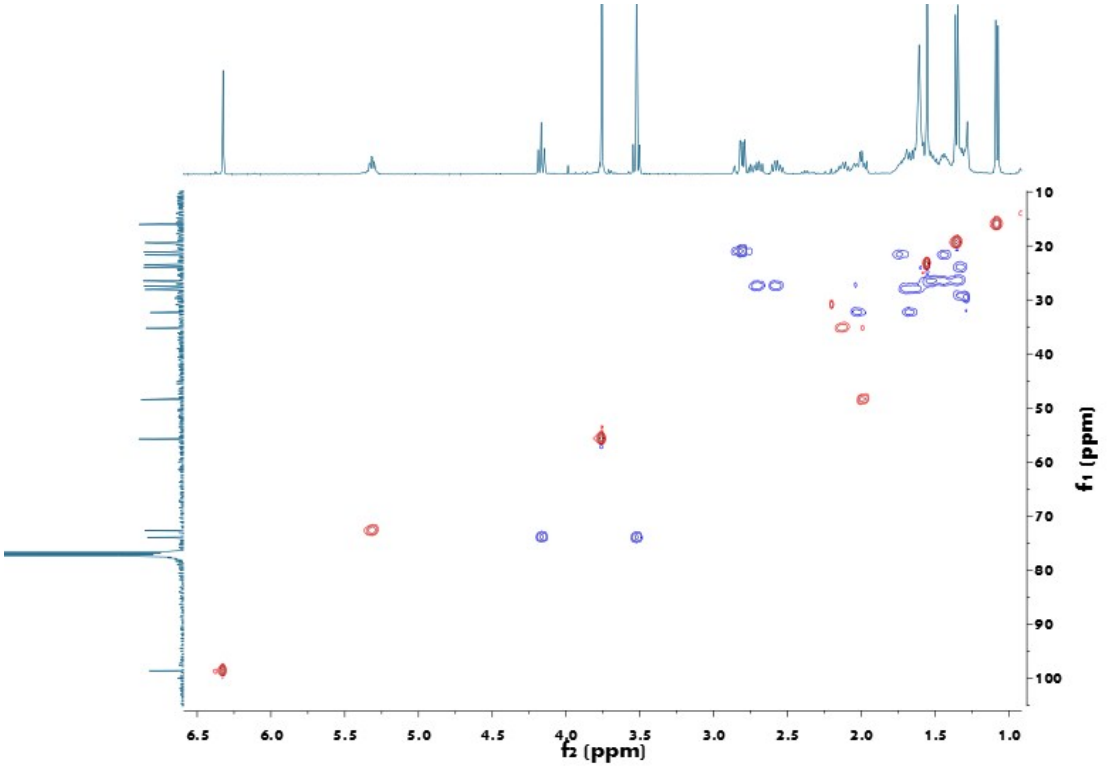


Fig. S5 ^{1}H-^{1}H COSY spectrum of **1** in $CDCl_3$

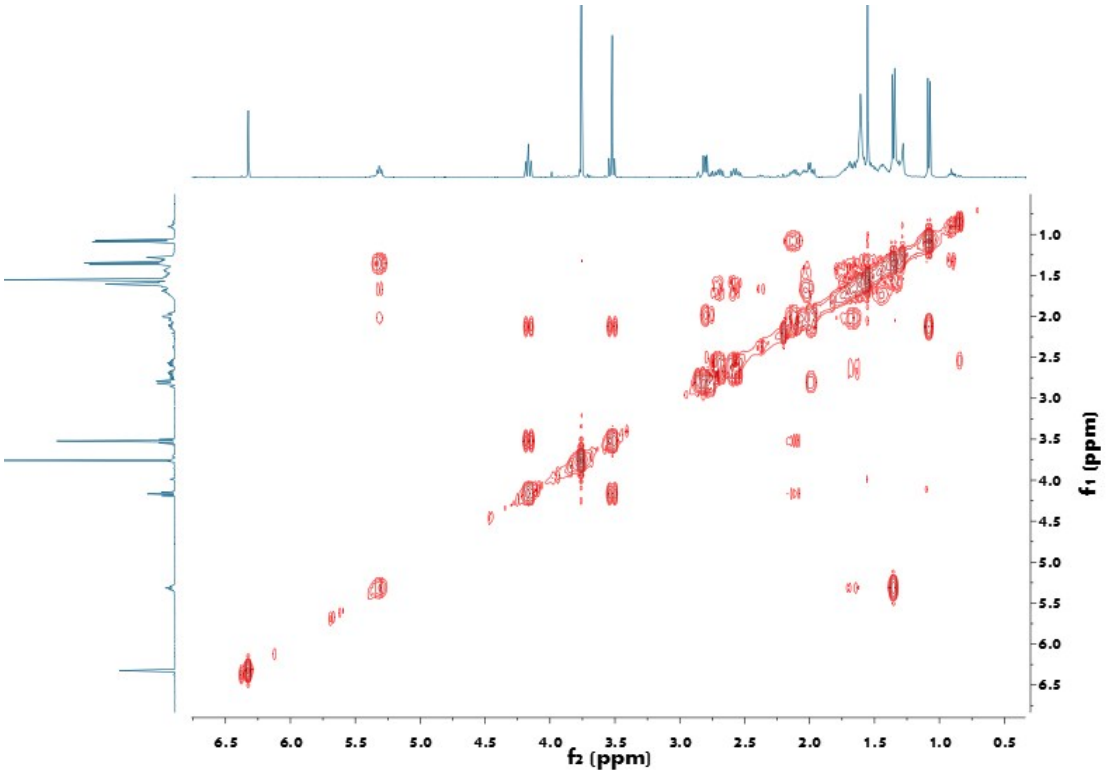


Fig. S6 HMBC spectrum of **1** in $CDCl_3$

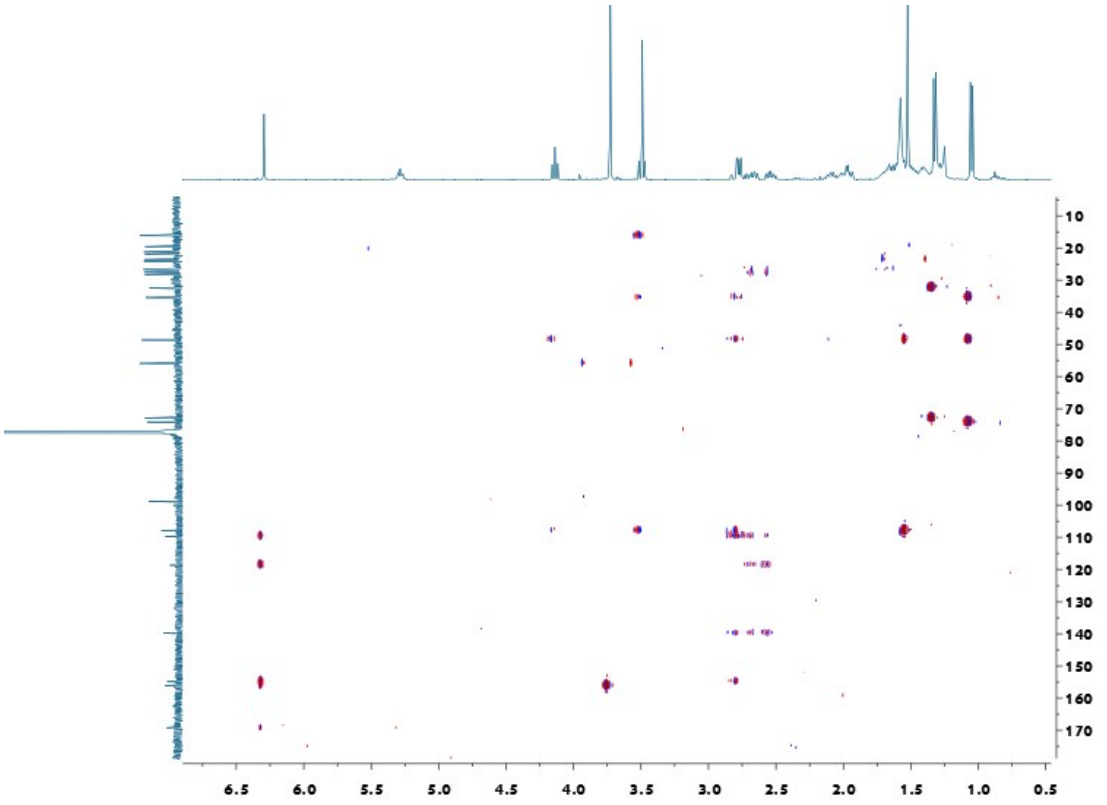


Fig. S7 NOESY spectrum of **1** in $CDCl_3$

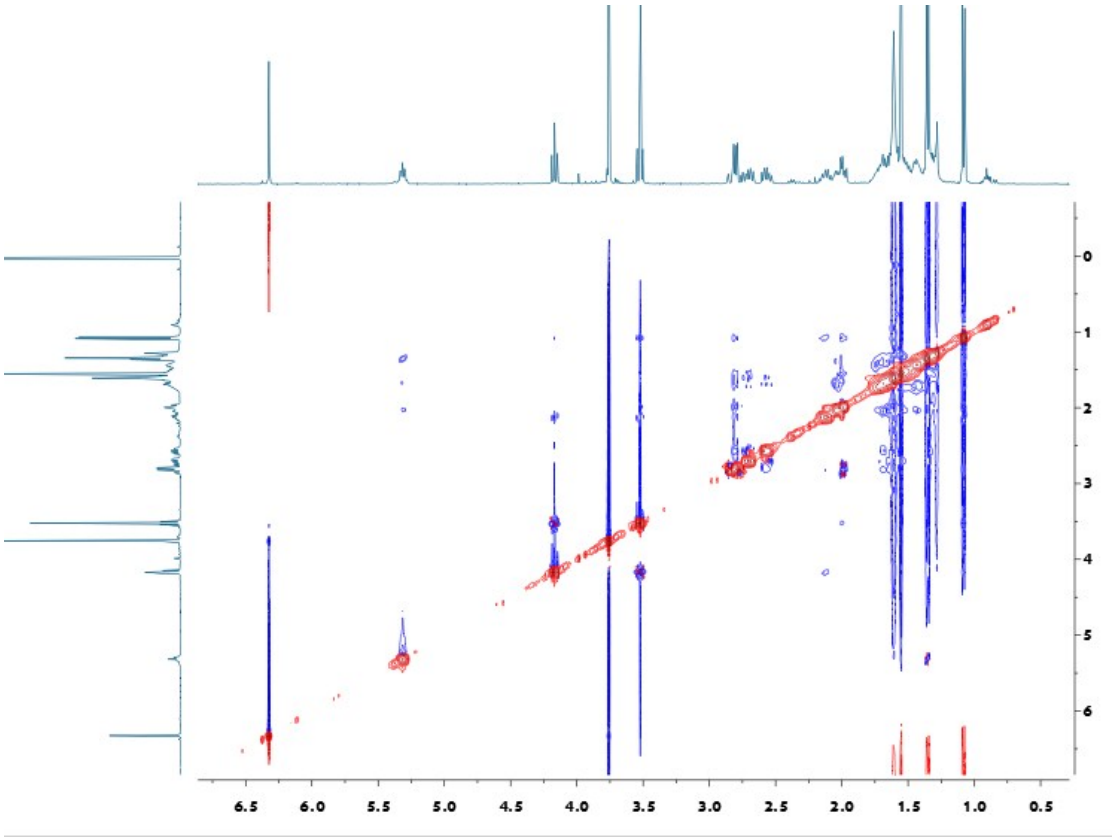
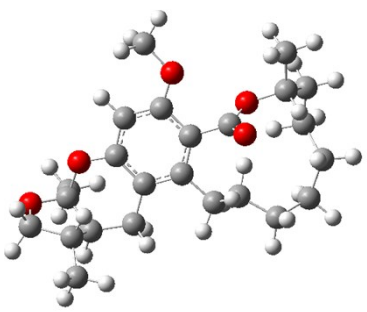


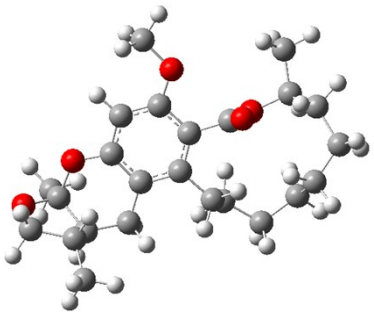
Table S1. Energy Analysis for the Conformers of (15*R*,18*S*,19*S*,21*S*)-**1**.

compound	Conformation	G (Hartree)	G (Kcal/mol)	ΔG (Kcal/mol)	Boltzmann Dist (%)
(15*R*,18*S*,19*S*,21*S*)-**1**	**1a**	-1311.51863061	-822981.7441	0	54.83%
(15*R*,18*S*,19*S*,21*S*)-**1**	**1b**	-1311.51782715	-822981.2399	0.50417348	23.40%
(15*R*,18*S*,19*S*,21*S*)-**1**	**1c**	-1311.51752349	-822981.0494	0.694721011	16.96%
(15*R*,18*S*,19*S*,21*S*)-**1**	**1d**	-1311.51633526	-822980.3038	1.440338782	4.81%

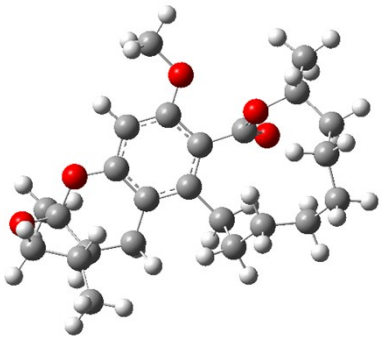
Figure S8. B3LYP/6-31G(d) optimized low-energy conformers of **1**



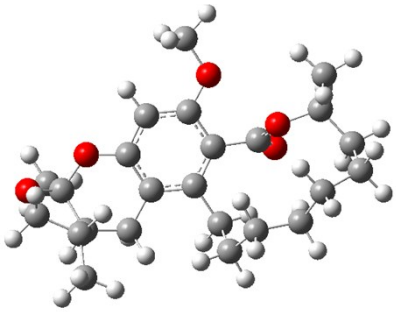
1a



1b



1c



1d

References.

Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, revision C.01. Gaussian, Inc.: Wallingford CT, 2010.Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Bringmann, G. SpecDis: Quantifying the comparison of calculated and experimental electronic circular dichroism spectra. Chirality 2013, 25, 243–249.

Experimental Section

1. General Experimental Procedures

The NMR spectra were recorded on Bruker Avance Bruker Avance 500 spectrometer (^{1}H 500 MHz, ^{13}C 125 MHz). All chemical shifts (δ) were given in ppm with reference to the solvent signal (δ_C 39.52/δ_H 2.50 for DMSO), and coupling constants (J) were given in Hz. UV data was obtained on a PERSEE TU-1900 spectrophotometer. IR spectrum were performed on a Nicolet Nexus 670 spectrophotometer, in KBr discs. HRESIMS spectrum was recorded on a Shimadzu LCMS-IT-TOF mass spectrometer. Column chromatography (CC) was performed on silica gel (200-300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Pharmacia, Piscataway). Precoated silica gel plates (Qingdao Huang Hai Chemical Group Co., G60, F-254) were used for thin layer chromatography.

2. Fungus Material.

The fungus ZJ-HQ1 used in this study was isolated from healthy leaf of the marine mangrove *A. ilicifolius*, which was collected in April 2012 from Zhanjiang Mangrove Nature Reserve in Guangdong Province, China. The fungus was obtained using the standard protocol for isolation.[27] Fungal identification was carried out using a molecular biological protocol by DNA amplification and sequencing of the ITS region. The sequence data obtained from the fungal strain have been deposited at GenBank with accession no. KT240140. A BLAST search result showed that the sequence was the most similar (99%) to the sequence of *L. theobromae* (compared to JQ658976.1 HM466955.2). A voucher strain was deposited in the China Center for Type Culture Collection under patent depository number CCTCC M 2016219.

3. Fermentation, Extraction and Isolation.

The fungus was cultured on autoclaved rice solid-substrate media (sixty 500 mL Erlenmeyer flasks; each containing 50 g of rice, 1.5 g of artificial sea salts, and 50 mL of distilled H_2O) at room temperature under static conditions and daylight for 28 days. Following incubation, the mycelia and solid rice media were extracted with EtOAc.

The extract was evaporated under reduced pressure to yield 268g of residue. The residue was then divided into 36 fractions (Fr. 1–Fr. 36) by column chromatography on silica gel, eluting with a gradient of petroleum ether/EtOAc from 1:0 to 0:1. Fr. 18 (309 mg) was subsequently separated by Sephadex LH-20 CC eluting with CH_2Cl_2/MeOH (v/v, 1:1) to give subfraction Fr. 18.6, which was purified on silica gel (CH_2Cl_2/MeOH v/v, 99:1) to yield compound **1** (1.6 mg).

*4. Spectroscopic Data of Compound **1**.*

Compound **1**: white powder; $[\alpha]_D^{20}$ +33 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 207 (4.3), 288 (3.3) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$): 205 (+16.7), 248 (−8.2) nm; IR (KBr) ν_{max} 2923, 2852, 1720, 1600, 1464, 1384, 1338, 1270, 1201, 1103, 1004, and 875 cm^{-1}; EIMS *m*/*z* 402 $[M]^+$, HREIMS *m*/*z* 402.2397 ($[M]^+$, calcd for $C_{24}H_{34}O_5$, 402.2401); for ^1H and ^{13}C NMR data, see Table 1.

5. Computational details

Molecular Merck force field (MMFF) and DFT/TD-DFT calculations were carried out with Spartan' 14 software (Wavefunction Inc., Irvine, CA, USA) and Gaussian 09 program, respectively. Conformers were generated and optimized using DFT calculations at B3LYP/6-31G(d) level. Conformers with Bolzmann distribution over 1% were chosen for ECD calculations in methanol at B3lYP/6-311+g(2d,p) level. The IEF-PCM solvent model for MeOH was used. ECD spectra were generated using the program SpecDis 3.0 (University of Würzburg, Würzburg, Germany) and OriginPro 8.5 (OriginLab, Ltd., Northampton, MA, USA) from dipole-length rotational strengths by applying Gaussian band shapes with sigma = 0.30 ev. All calculations were performed by Tianhe-2 in National Super Computer Center in Guangzhou.

6. Bioactivity Assay.

6.1 Cell Culture, Measurement of NO Production, and Cell Viability.

RAW264.7 cells were purchased from Cell bank of Chinese Academy of Sciences (Shanghai, People's Republic of China). Cell maintenance, experimental procedures,

and data determination for the inhibition of NO production and the viability assay are the same as literatures[7]. The IC_{50} values were determined using Origin 8 Pro. software from experiments performed in triplicate. Indomethacin (IC_{50} value of 26.3 ± 1.2 μM) was used as a positive control. All tested compounds were prepared as stock solutions in DMSO and final solvent concentration was less than 0.2% in all assays.

6.2 Cytotoxicity Assay.

MCF-7, HEK293T, HeLa, HepG2 cells were cultured in Dulbecco's modification Eagles's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), 50 U/mL penicillin (Gibco) and 50 mg/mL streptomycin (Gibco).[28] A549 cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI-1640, Gibco) supplemented with 10% FBS (Gibco). The cells were incubated at 37 °C containing 5% CO_2.

MCF-7, HEK293T, HeLa, HepG2, A549 cells were seeded in 96-well plates (NUNC) at a density of 5×10^3 cells per well and incubated overnight. Then the cells were treated with various concentrations of compounds for 72 h. Subsequently, 10 μL of MTS solution (CellTiter 96Aqueous One Solution Reagent, Promega) were added to each well, and the cells were incubated for 1 h at 37°C. The absorbance was measured at 490 nm by Infinite M200PRO microplate reader (TECAN). The negative control was defined as cells that were treated with 1% DMSO. The background absorbance was corrected by the blank control containing culture medium and MTS. Cell viability was calculated using the following formula: Cell viability/% = (Absorbance of treated cells – Absorbance of blank control)/(Absorbance of negative control – Absorbance of blank control) × 100. The half maximal inhibitory concentration (IC_{50}) was calculated with Origin 8 Pro. All assays were performed in triplicates in three independent experiments.

6.3 α-Glucosidase inhibitory activity

The assay of α-glucosidase inhibitory activity was carried out under 0.01 M potassium phosphate buffer (pH 7), using a microtiter plate reader. Enzyme solutions were prepared to give 2.0 units/mL in 2 mL buffer solution. Diluted enzyme solution (10 μL), test samples (20 μL, in DMSO) and buffer solution (145 μL) were mixed in each well of a 96-well microtiter plate. After pre-incubated for 20 min at 37 °C, PNPG (25

μL, 3 mg/mL) was added to start the enzymatic reaction measured by a microtiter plate reader at 410 nm in 37 °C immediately. Calculations were performed according to the equation: η(%) = [(B – ¬S) / B] × 100% (B stands for the assay medium with DMSO, S stands for the assay medium with inhibitor). All measurements were done in triplicate from two independent experiments. The reported IC_{50} was the average value of two independent experiments.