

Cytotoxic and antiviral nitrobenzoyl sesquiterpenoids from the marine-derived fungus *Aspergillus ochraceus* Jcma1F17

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Experimental procedures of bioactive assays.

Cytotoxic bioassay. Ten human cancer cell lines (H1975, U937, K562, BGC-823, Molt-4, MCF-7, A549, HeLa, HL60, and Huh-7) were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. Cells were routinely grown and maintained in RPMI or DMEM medium with 10% FBS and 1% penicillin/streptomycin. All cell lines were incubated in a Thermo/Forma Scientific CO₂ water-jacketed incubator with 5% CO₂ in air at 37 °C. Cell viability assay was determined by CCK-8 (Dojindo, Japan) assay. The cells were seeded at a density of 400 to 800 cells/well in 384 well plates and then treated with various concentrations of compounds or solvent control. After 72 h of incubation, CCK-8 reagent was added, and absorbance was measured at 450 nm by an Envision 2104 multi-label reader (Perkin Elmer, USA). Dose response curves were plotted to determine IC₅₀ using Prism 5.0 (GraphPad Software Inc., USA).

Antiviral bioassay against influenza virus H3N2. Cell-based anti-influenza virus inhibition screening was based on the principle of cytopathic effect (CPE) protection assay. The Madin–Darby canine kidney (MDCK) cells cultured to approximately 90% confluence were detached with 0.25% Trypsin–EDTA (Invitrogen), washed and re-suspended in complete EMEM, 2.5×10^4 MDCK cells were plated in triplicate in a 96-well plate and incubated overnight at 37 °C in a humidified 5% CO₂ incubator. The confluent MDCK monolayers cells were rinsed twice with Hanks' solution devoid of serum, and then the cells were treated with 50 µL medium with 1 mg/mL

TPCK and 0.3% BSA and infected by H3N2 virus at a multiplicity of infection (MOI) of 0.01 PFU/cell, then incubated in 37 °C for 2 h, serially diluted compounds were added. After 3 days incubation, the medium was removed and 50 µL medium containing 5 µL CCK-8 reagent was added into each well followed by additional 2 h incubation, the absorbance was measured at 450 nm using an UVstar-Microplates Synergy HT plate reader. The IC₅₀ values were calculated by nonlinear regressions using GraphPad Prism 5.

Antiviral bioassay against EV71 virus. This assay measured the ability of a test compound to inhibit the CPE induced by EV71 virus on Vero cells with CCK-8 assay. Vero cells (2×10^3 cells/well) were seeded with DMEM medium (2% FBS) into a 384-well plate. After 24 h, 1 000 fold serial dilution of the compound was added in triplicate to the 384-well plate. After incubation at 37 °C for 30min, a two folds dilution $100 \times$ the 50% tissue culture infectious dose (TCID₅₀) of EV71 virus in DMEM supplemented with 2% FBS was added to each well. The plate was incubated at 37 °C for 72–96 h when the viral control cells showed complete CPE, the cell survival was quantified using CCK-8. The A450 of the well was measured with a microtiter plate reader (Envision, PerkinElmer). The IC₅₀ value of the testing compound was calculated using the GraphPad Prism software.

Antituberculosis bioassay. Antituberculosis was assayed as reported.^{1,2}

Autoluminescent *Mycobacterium tuberculosis* H37Ra was inoculated in a 50 mL centrifuge tube containing 5 mL of 7H9 broth (Becton Dickinson) with 0.1% Tween

80 and 10% OADC enrichment (Becton Dickinson) and then incubated at 37 °C.

When the cultures reached an OD₆₀₀ nm of 0.3 to 1.0, the culture was diluted, and 50 µL diluted H37Ra was inoculated in sterile 384 well plates, the RLU of which should be between 10 000 and 50 000 and should be recorded as the base luminescent Day0. The compounds and the positive drug (rifampicin) were added to the 384 well plates in triplicate with a final concentration of 0.1 µM to 30.0 µM. The luminescent value was detected for the following 3 d. The data were analyzed with Excel relative to the DMSO control to estimate the inhibitory activity of the compounds.

REFERENCES

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Proc. Natl. Acad. Sci. USA **2002**, *99*, 3920-3925
- (2) Changsen, C.; Franzblau, S. G.; Palittapongarnpim, P. *Antimicrob. Agents Chemother.* **2003**, *47*, 3682-3687

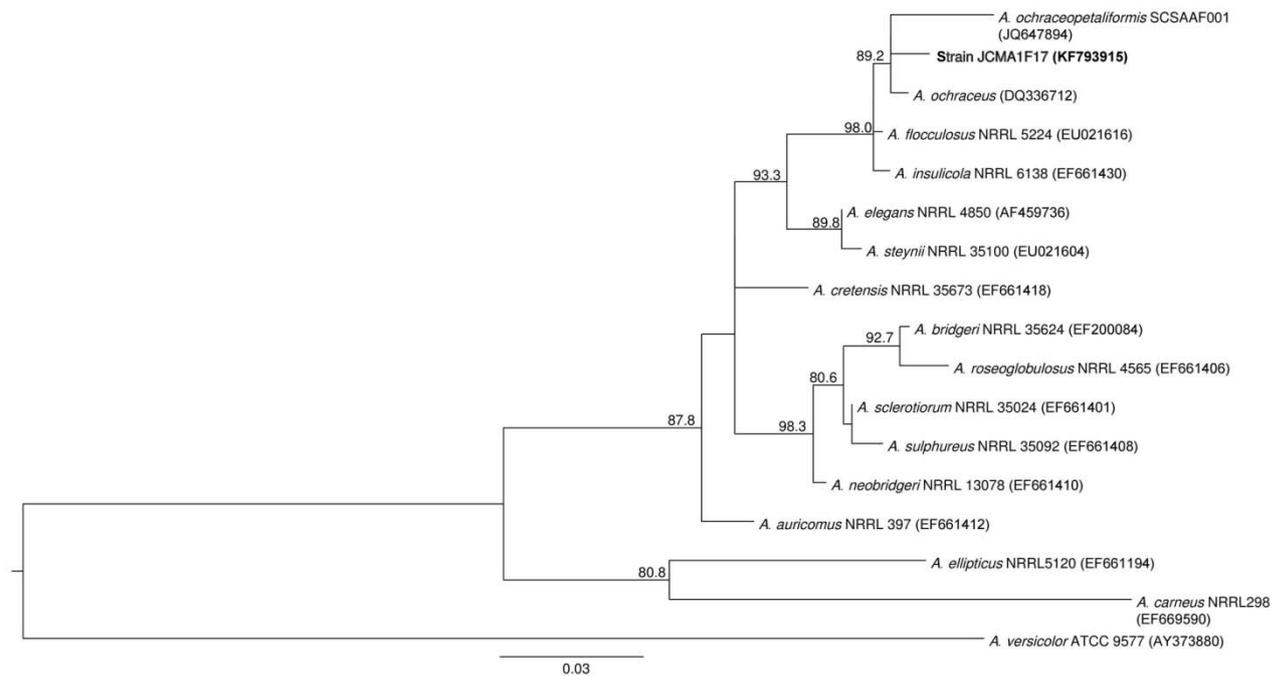


Figure S1. The maximum-likelihood tree based on ITS1-5.8S-ITS2 sequences, showing phylogenetic relationship between strain Jcma1F17 and related *Aspergillus* species. Only bootstrap values > 70% are shown. GenBank accession numbers are given in *parentheses*. Bar: 3% sequence divergence.

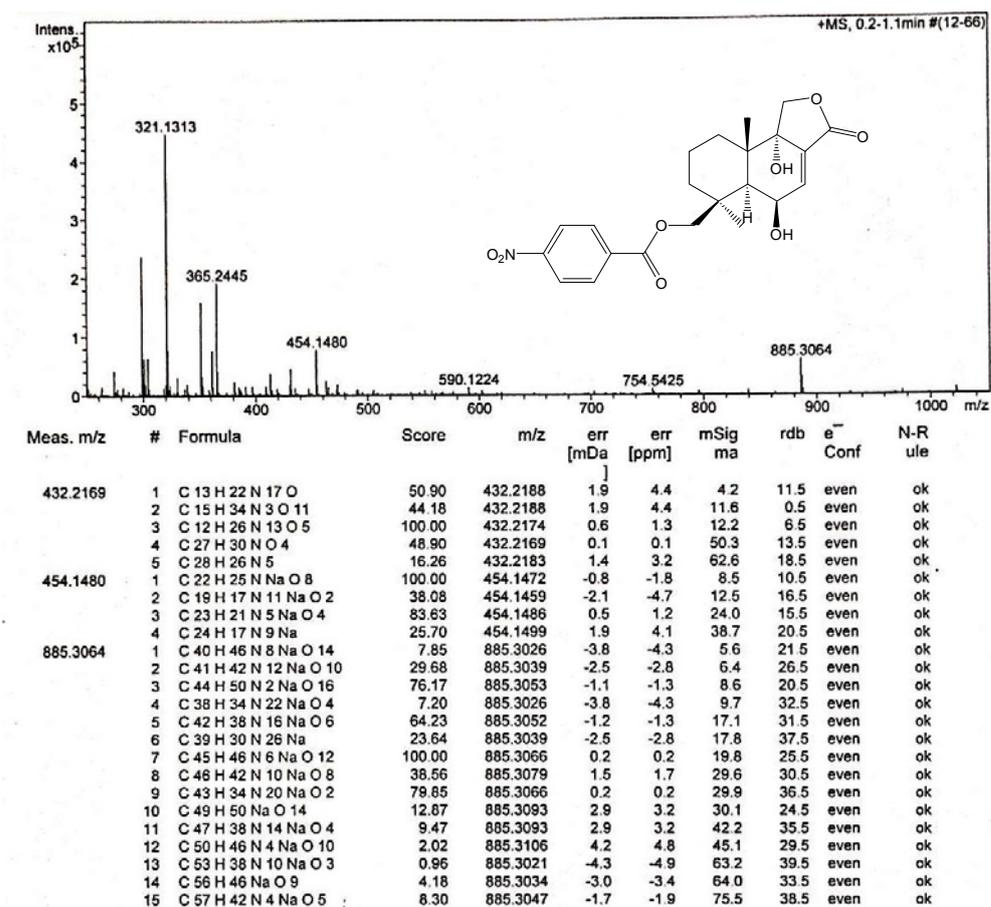


Figure S2 HRESI-MS spectrum of **1**

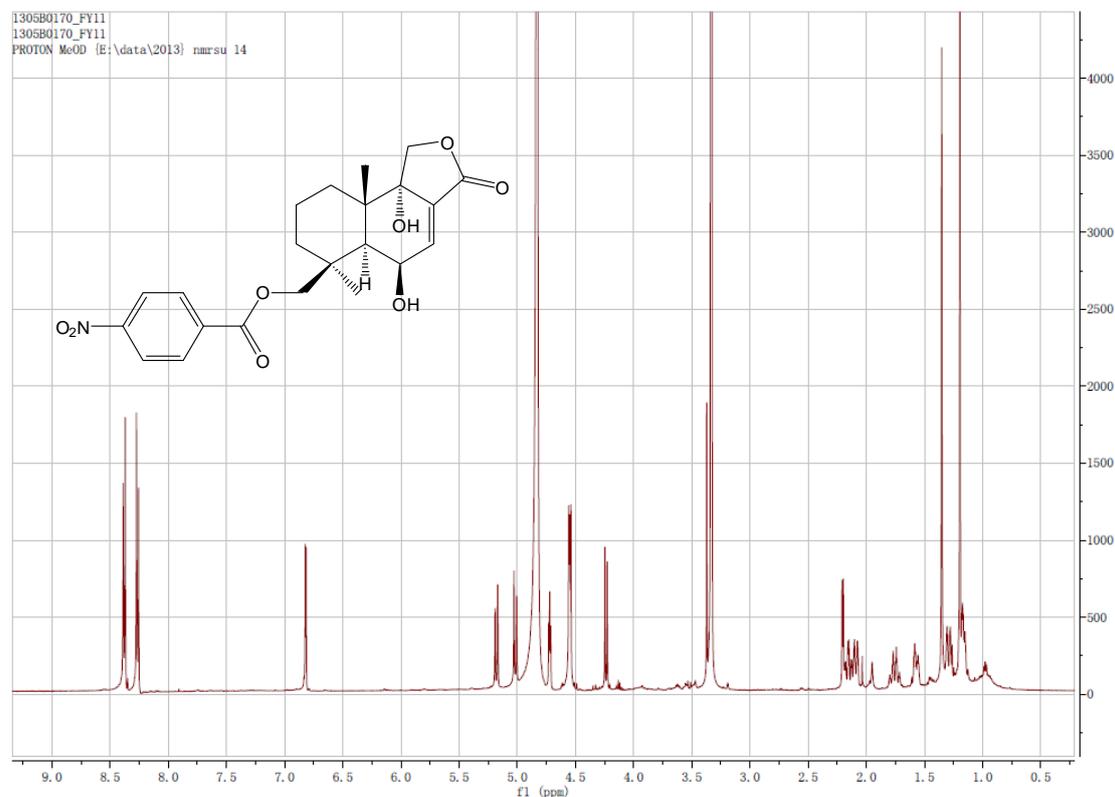
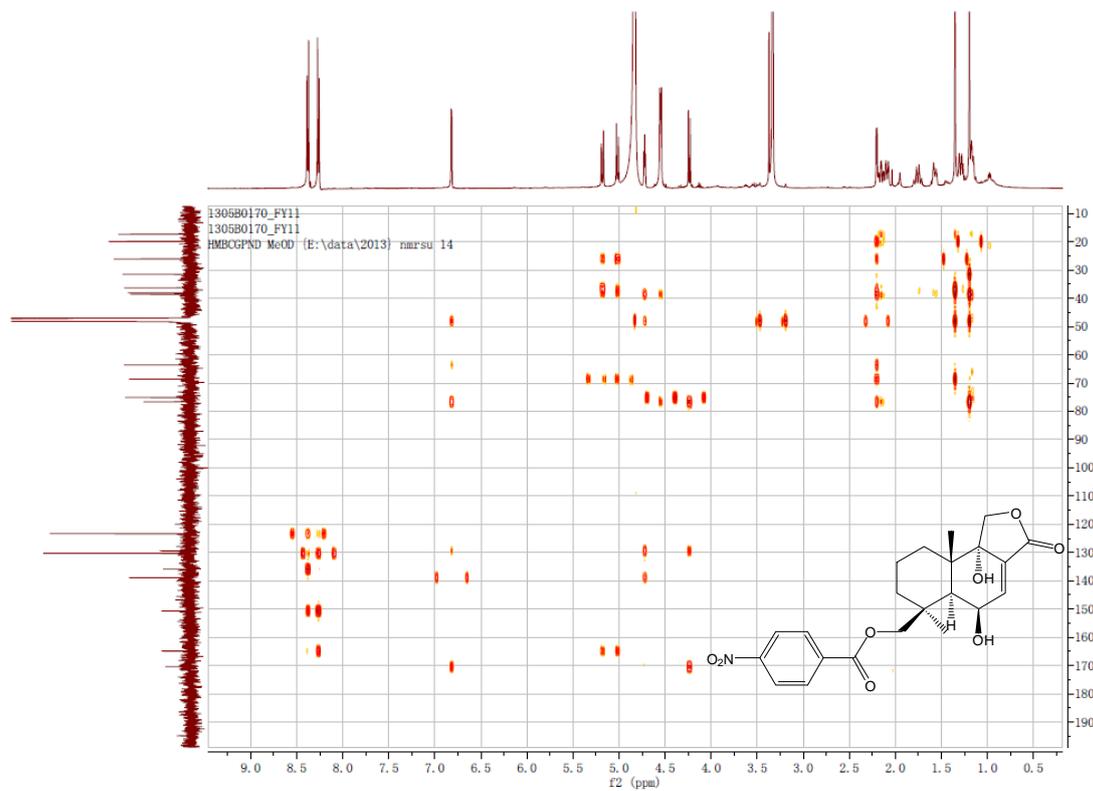
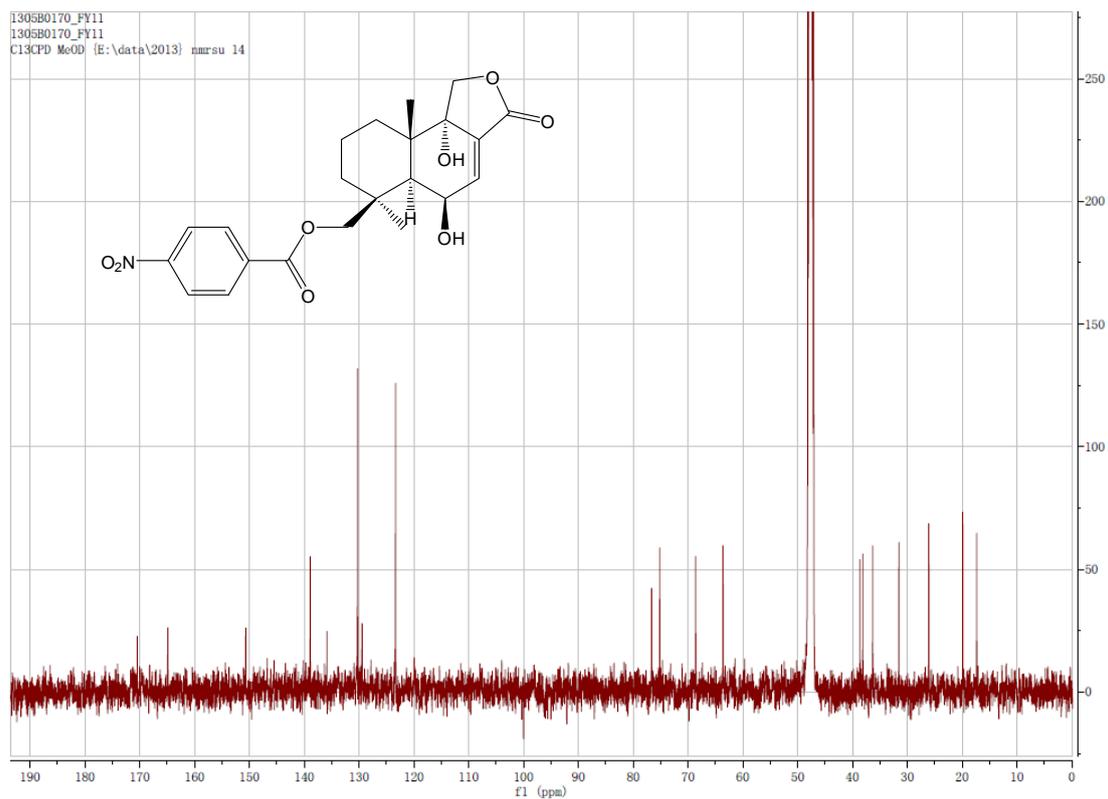


Figure S3 ¹H NMR spectrum of **1** in MeOD



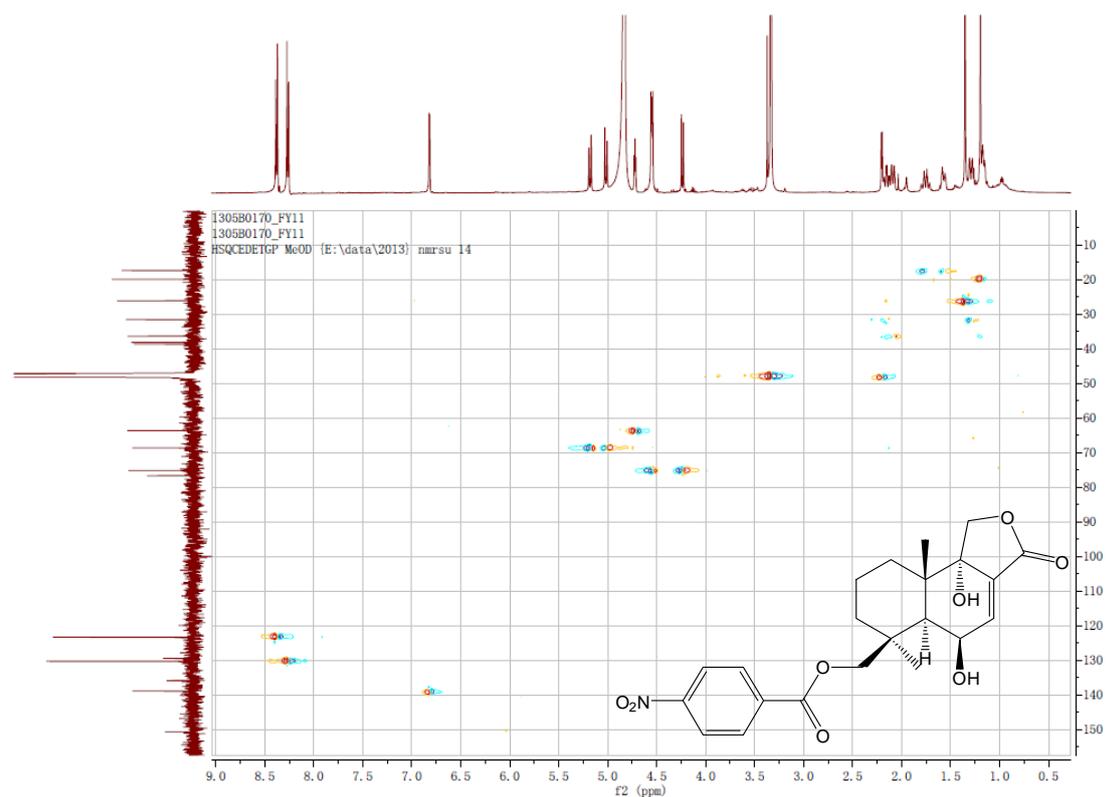


Figure S6 HSQC spectrum of **1** in MeOD

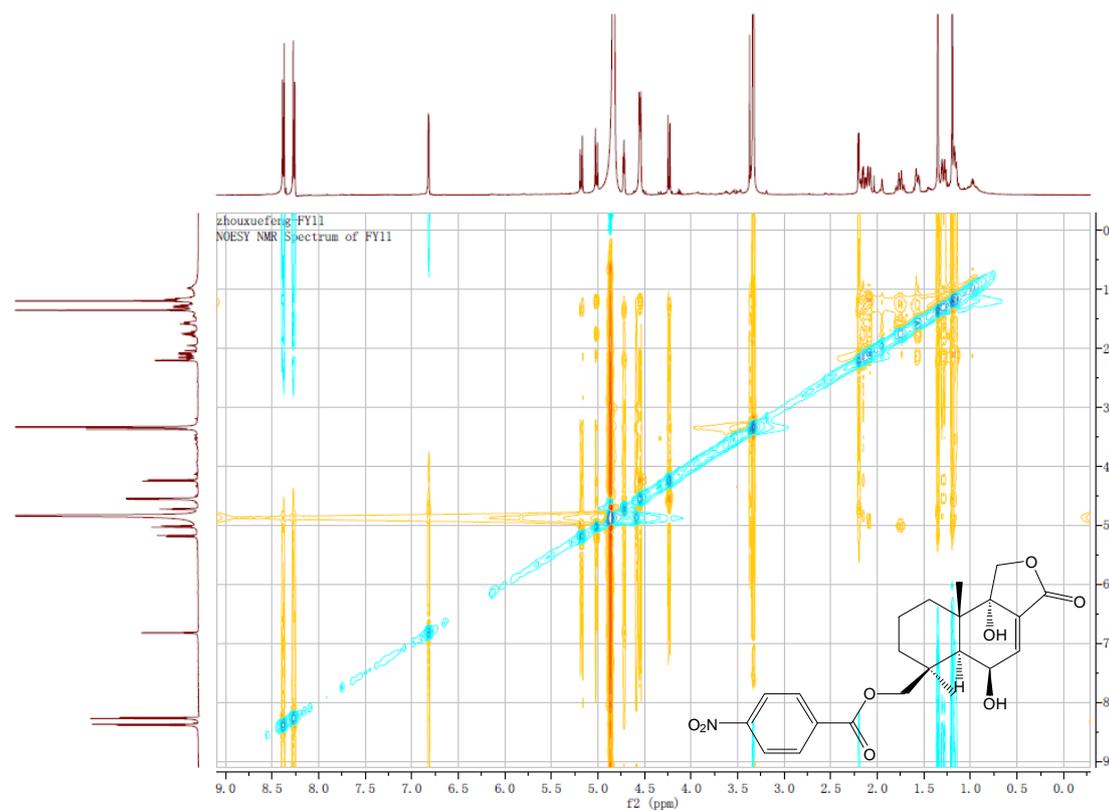


Figure S7 NOESY spectrum of **1** in MeOD