Supporting information

Asperterpenoid A, a New Sesterterpenoid as Inhibitor of *Mycobacterium tuberculosis* Protein Tyrosine Phosphatase B from the Culture of *Aspergillus* sp. 16-5c

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Contents

General Experimental Procedures.

General Experimental Procedures. Melting points were determined on an X-4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Polartronic HHW5 digital polarimeter. IR spectrum was measured on a Bruker Vector 22 spectrophotometer using KBr pellets. UV data was recorded on Shimadzu UV-2501PC. The NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$. All chemical shifts (δ) are given in ppm with reference to the solvent signal ($CDCl_3$, δ_H 7.26 for ^{1}H, δC 77.23 for ^{13}C), and coupling constants (*J*) are given in Hz. LRESIMS spectra were recorded on a Finnigan LCQ-DECA mass spectrometer. HRESIMS spectra were recorded on a Shimadzu LCMS-IT-TOF mass spectrometer. Single-crystal data were measured on an Oxford Gemini S Ultra diffractometer. Column chromatography (CC) was performed on silica gel (200-300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Pharmacia).

Fungal Material. The fungus used in this study was isolated from a mangrove endophytic fungus from the leaves of S. apetala, which were collected in Hainan Island, China. The fungus was identified as *Aspergillus sp.* by the ITS region (deposited in GenBank, accession no JX993829). A voucher strain was deposited in the China Center for Type Culture Collection under patent depository number CCTCC M 2012358

Fermentation and extraction. Spores of *Aspergillus* sp *16-5C* were directly spreaded into 4×500 mL Erlenmeyer flasks, each containing 200 mL of potato-liquid media composed of (g/L): glucose (20), NaCl (30), the final pH was adjusted to 6.9, Four Erlenmeyer flasks of the inoculated media were incubated at 25 ℃ on a rotary shaker at 180 rpm for 72 h to yield the seed liquid.

The rice solid-substrate medium was carried out in 50 Erlenmeyer flasks (1000 mL) each containing 100 g of rice, 20 ml 3% sea salt liquid, all were prepared by autoclaving under 120℃ temperature about 30 minutes and Cooling to room temperature.

The seed liquid was transferred into the rice solid-substrate medium under aseptic conditions and incubating at 25 ℃ for 28 days. The mycelia and rice medium were extracted with MeOH. The MeOH layer was concentrated under reduced pressure to give a dark brown gum 6.8 g.

Materials and methods for mPTPB assay:

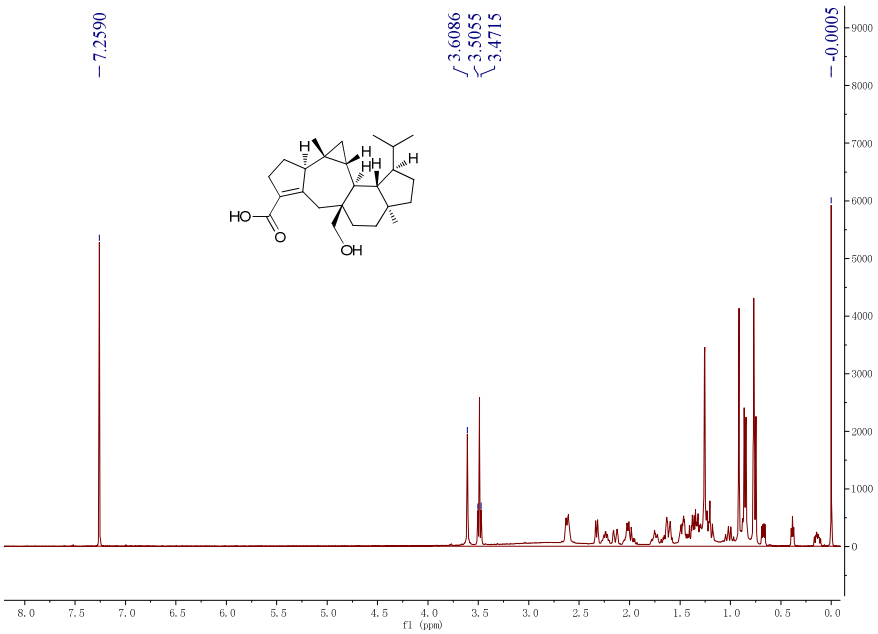
Cloning, Expression, and Purification of mPTPB

The full-length PTPB gene was amplified from genomic DNA of the *Mtb* H37Rv strain (School of Life Sciences, Sun Yat-sen University). PCR products were cloned in frame with an N-terminal His_6 tag into the pET28a (+) vector (Novagen). For protein expression, plasmids were transformed into *E. coli* BL21(DH3) cells (Invitrogen) and grown in LB medium containing 50 μg/ml kanamycin at 37°C till the OD_{600} of the solution were about 0.6. After addition of 0.1 mM IPTG, the culture was grown for another 16 hr at 20 ºC. The cells were harvested by centrifugation at 5,000 rpm for 5 min at 4 ºC. The bacterial cell pellets were resuspended in the buffer containing 20 mM Tris, pH 7.9, 500 mM NaCl, 5 mM imidazole and were lysed by sonication on ice. Cellular debris was removed by centrifugation at 16,000 rpm for 30 min at 4 ºC. The protein was purified from the supernatant using glutathione–Sepharose 4B (GE Healthcare) according to the manufacturer's instructions. Protein concentration was determined using the Bradford dye binding assay (Bio-Rad) according to the manufacturer's recommendations with bovine serum albumin as standard. The purified mPTPB were stored in 20% glycerol at -20°C.

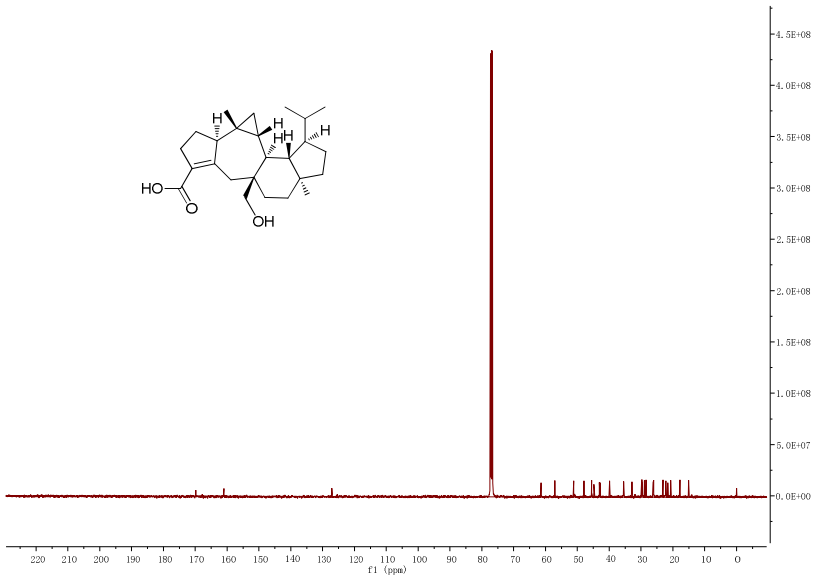
mPTPB Inhibition assay

The inhibition assays were performed using the RediPlate 96 EnzChek Tyrosine Phosphatase Assay kit (Invitrogen) by monitoring the hydrolysis of the fluorogenic phosphatase substrate, 6,8-difluoro-methylumbelliferyl phosphate (DiFMUP) according to the manufacturer's instruction. The IC_{50} value was determined at five different substrate concentrations by non-linear regression fitting of the inhibitor concentration versus inhibition rate. All measurements were done in triplicate from two independent experiments. The reported IC_{50} was the average value of two independent experiments.

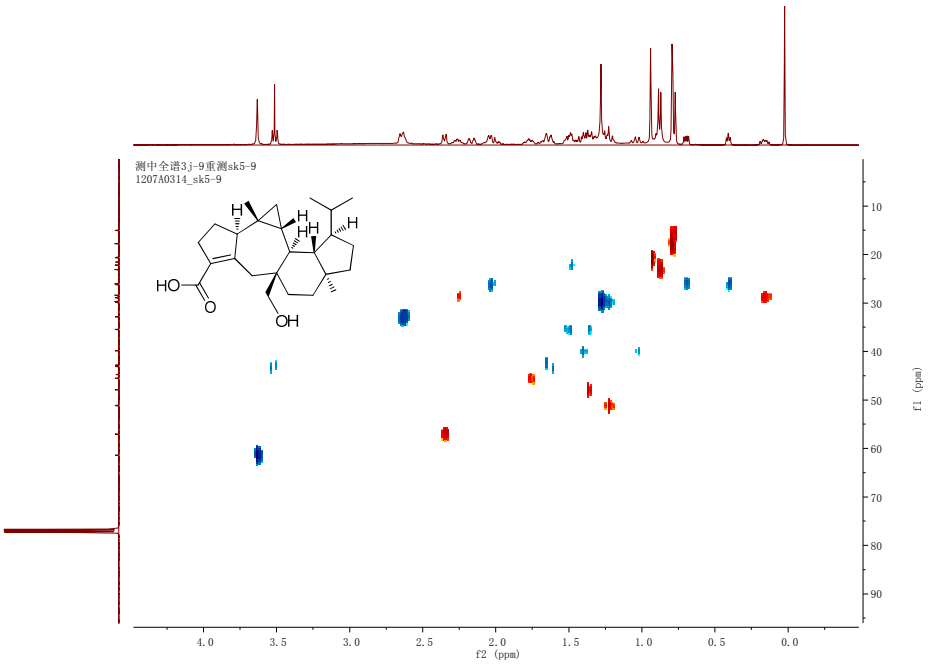
The ^{1}H NMR (400 MHz) spectrum of compound 1 in $CDCl_3$



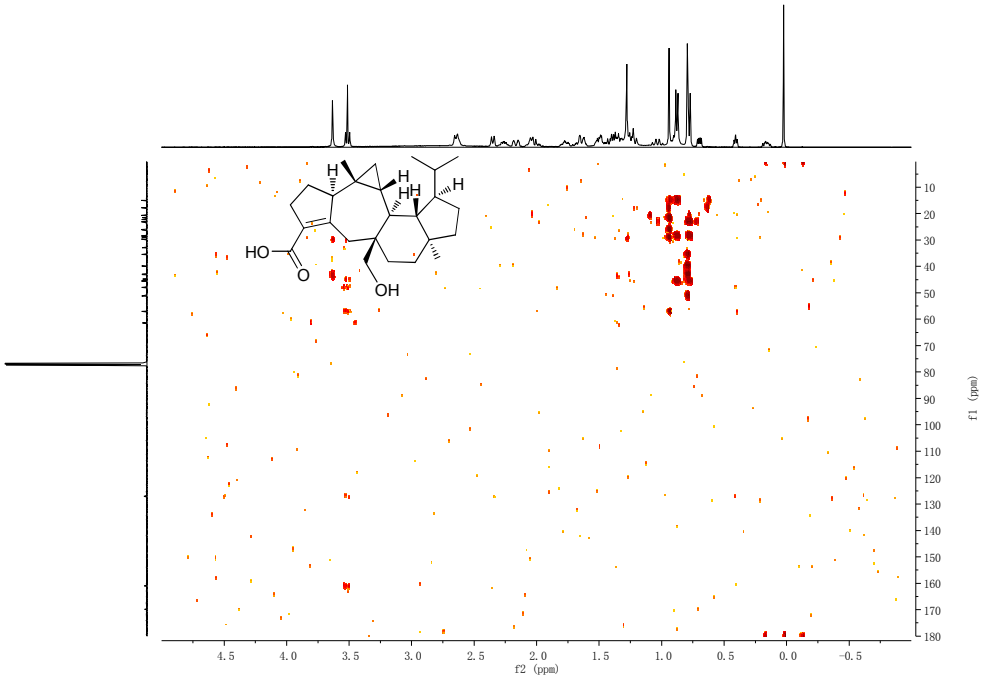
The ^{13}C NMR (100 MHz) spectrum of compound 1 in $CDCl_3$



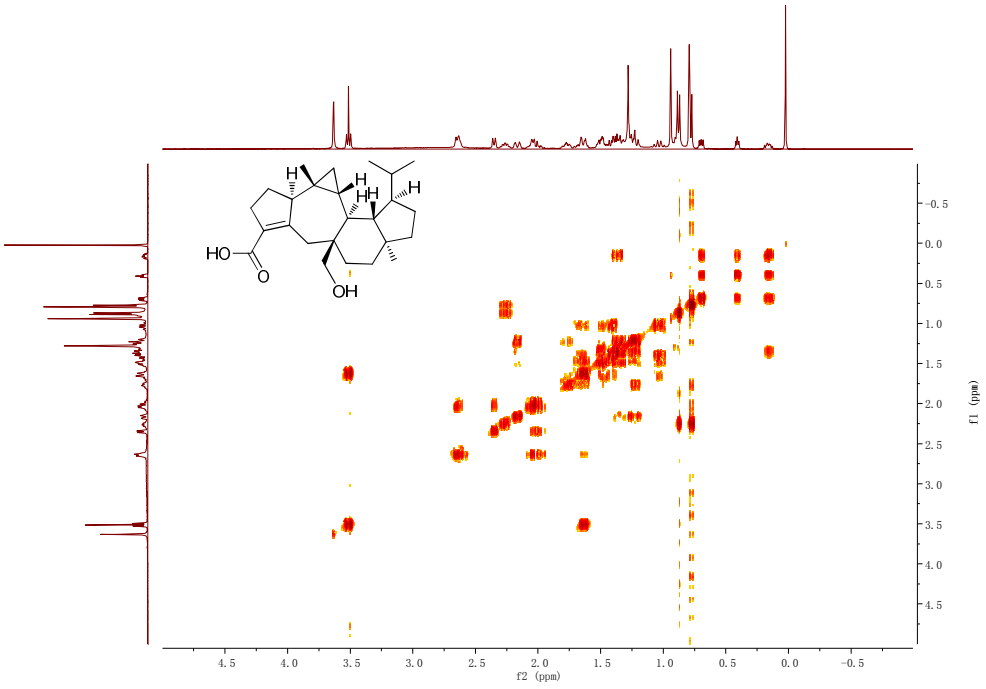
The HSQC (400 MHz) spectrum of compound 1 in $CDCl_3$



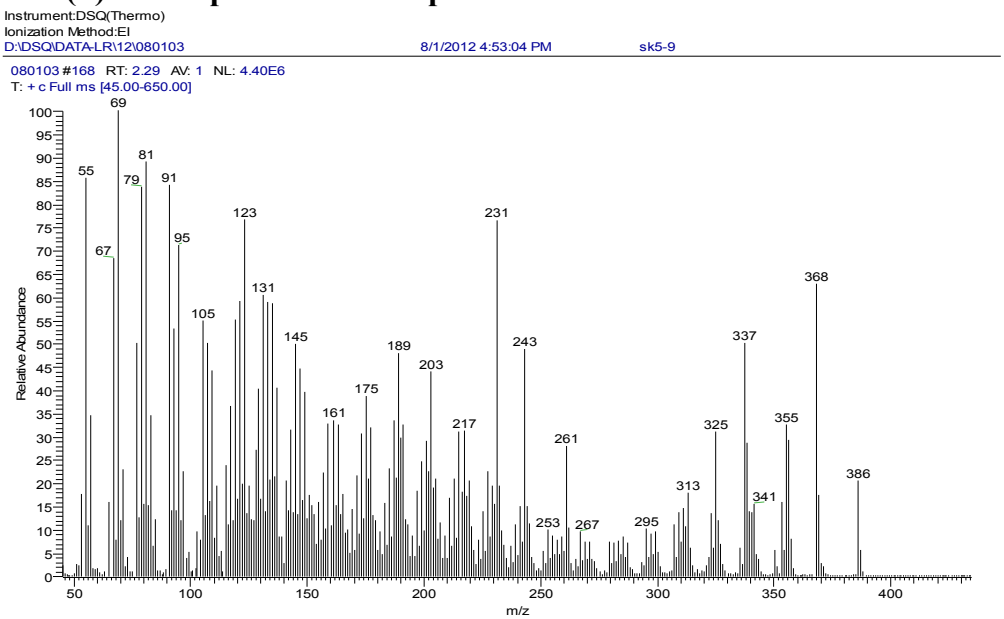
The HMBC (400 MHz) spectrum of compound 1 in $CDCl_3$



The ^{1}H-^{1}H COSY (400 MHz) spectrum of compound 1 in $CDCl_3$



The (+)EIMS spectrum of compound 1



The (+) HREIMS spectrum of compound 1

SPECTRUM - MS

Full ms [377.500 - 396.500] - Range: 386.000 - 386.300

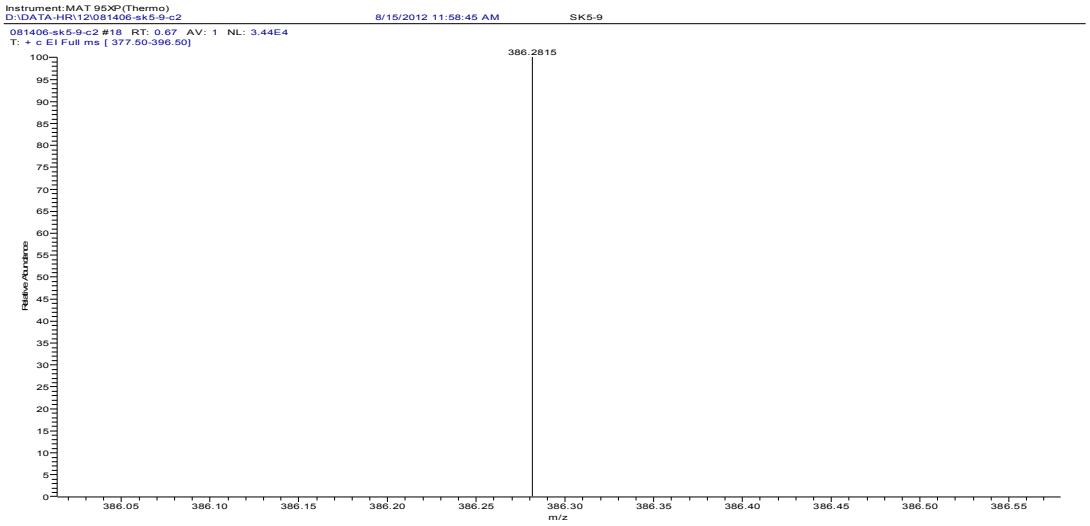
Scan No. 18 of 20

Scan #: 18

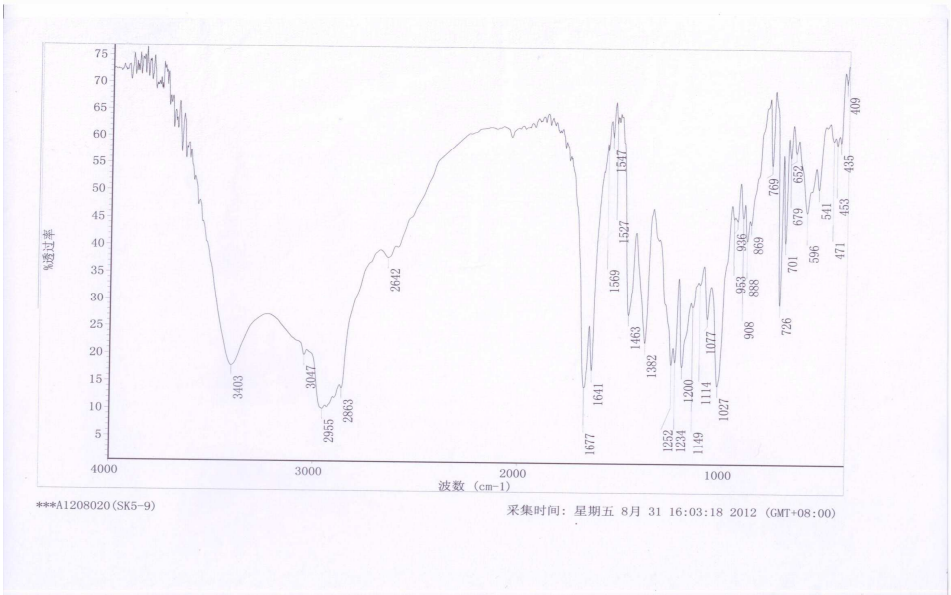
RT: 0.67

Data points: 1

Mass	Relative Intensity	Theoretical Mass	Delta	Delta [ppm]	RDB [mmu]	Composition
386.2815	40.8	386.2815	-0.1	-0.0	7.0	$C_{25}H_{38}O_3$



The IR spectrum of compound 1



The UV spectrum of compound 1in MeOH.