Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Anti-glioma trichobamide A with an unprecedented tetrahydro-5*H*-furo[2,3-b]pyrrol-5-one functionality from ascidian-derived fungus *Trichobotrys effuse* 4729

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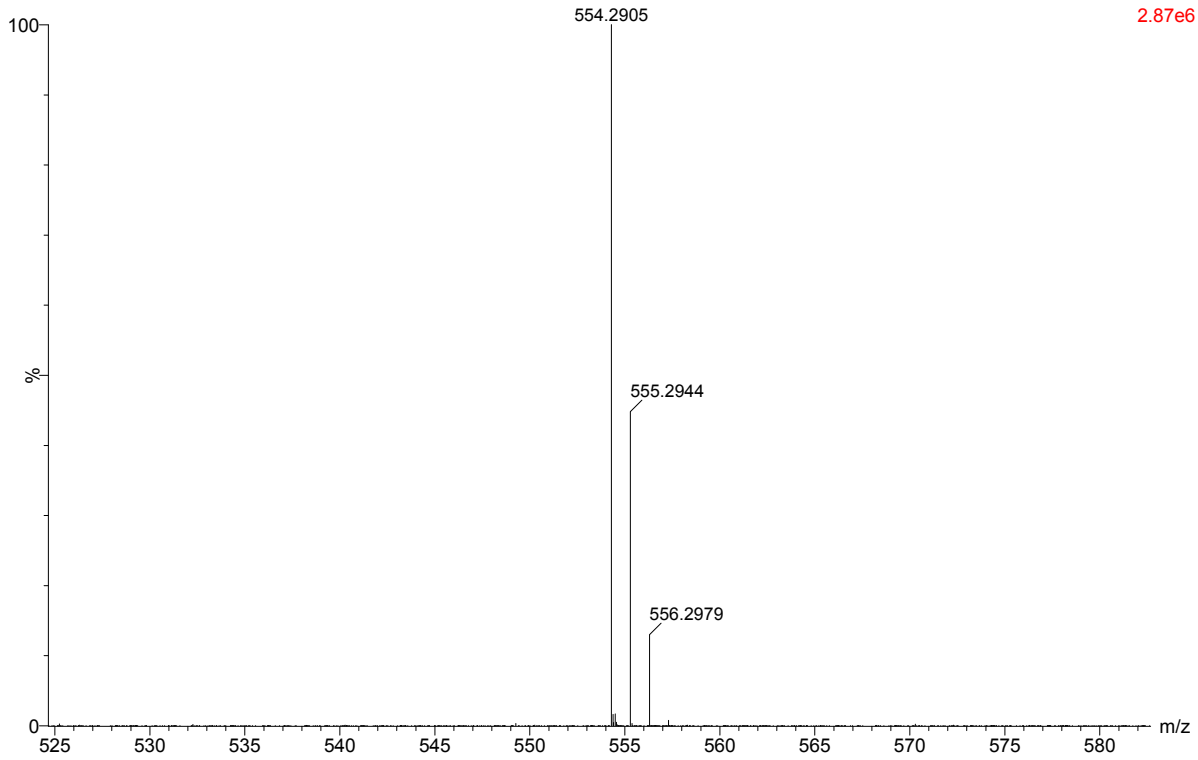
Fig. S1 HR−ESIMS spectrum of **1**



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
554.2905	554.2906	-0.1	-0.2	16.5	493.8	n/a	n/a	$C_{35}H_{40}NO_5$

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

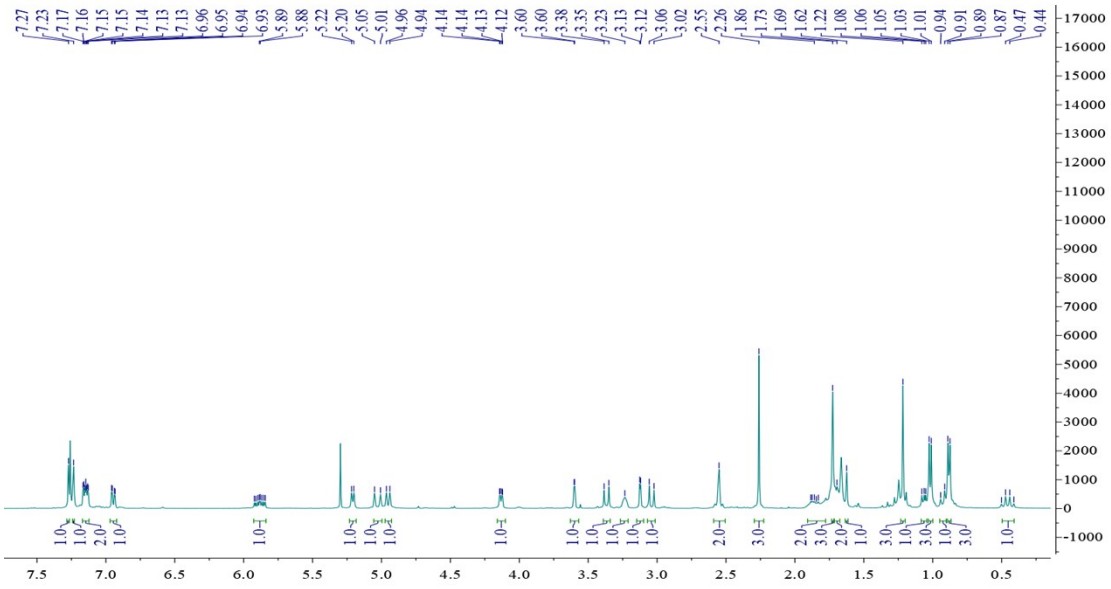


Fig. S3 ^{13}C NMR spectrum of **1** in $CDCl_3$

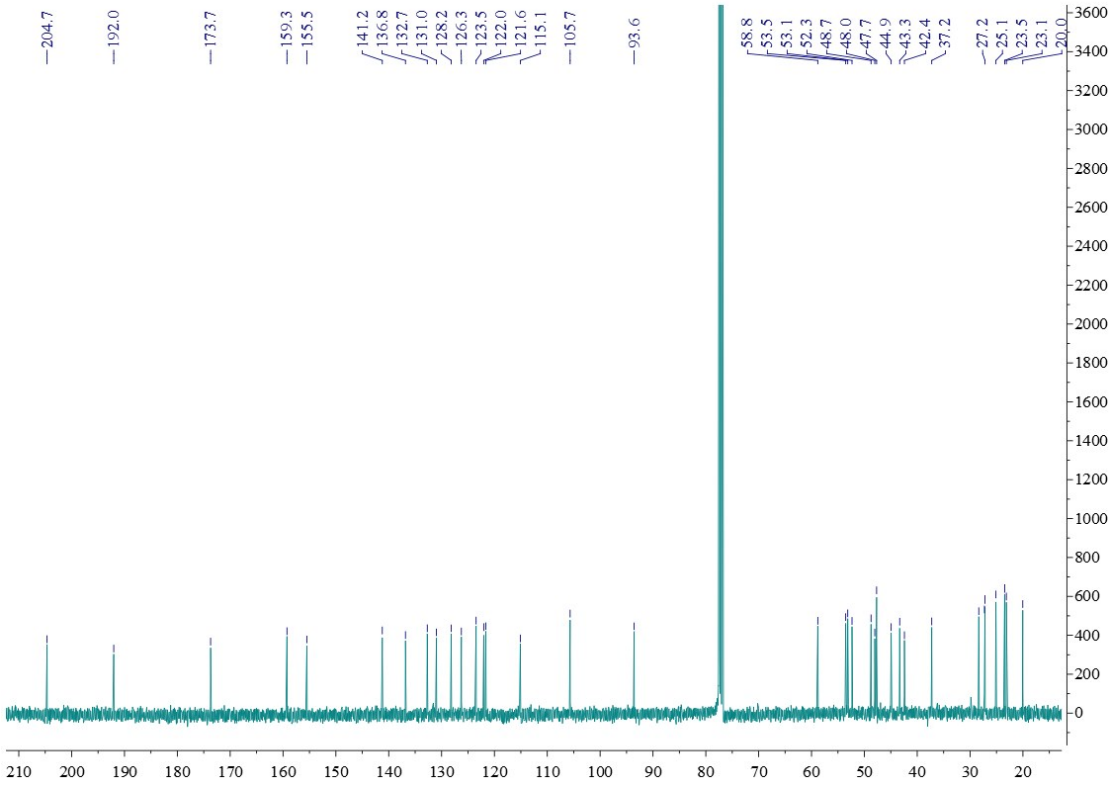


Fig. S4 DEPT-135 spectrum of **1** in $CDCl_3$

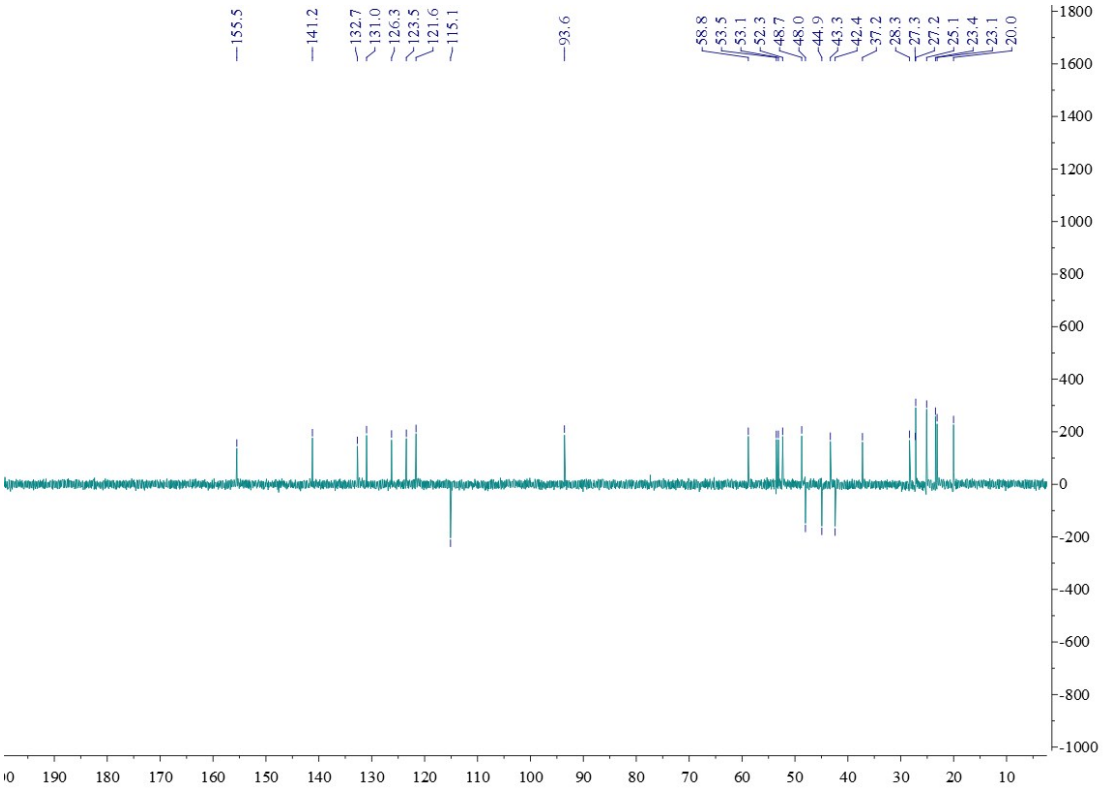


Fig. S5 HSQC spectrum of **1** in $CDCl_3$

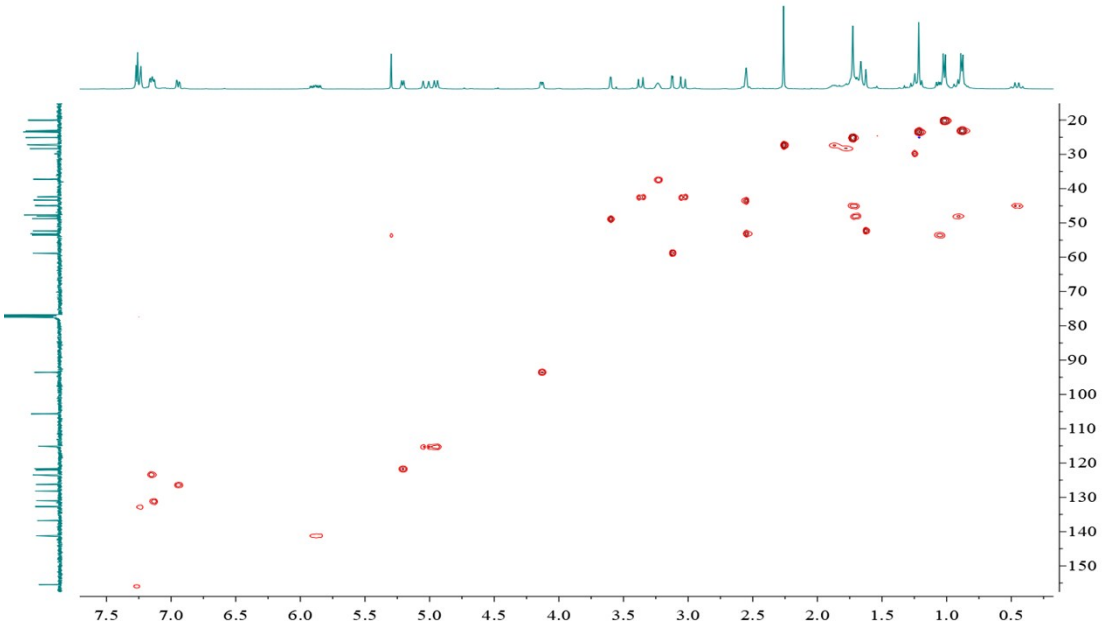


Fig. S6 ^{1}H-^{1}H COSY spectrum of **1** in $CDCl_3$

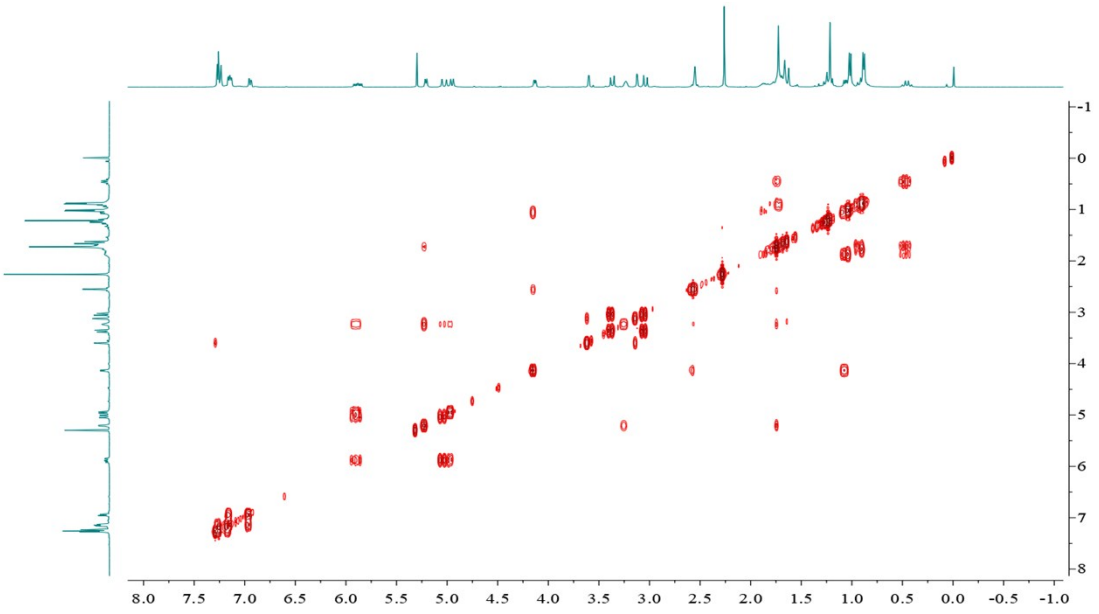


Fig. S7 HMBC spectrum of **1** in $CDCl_3$

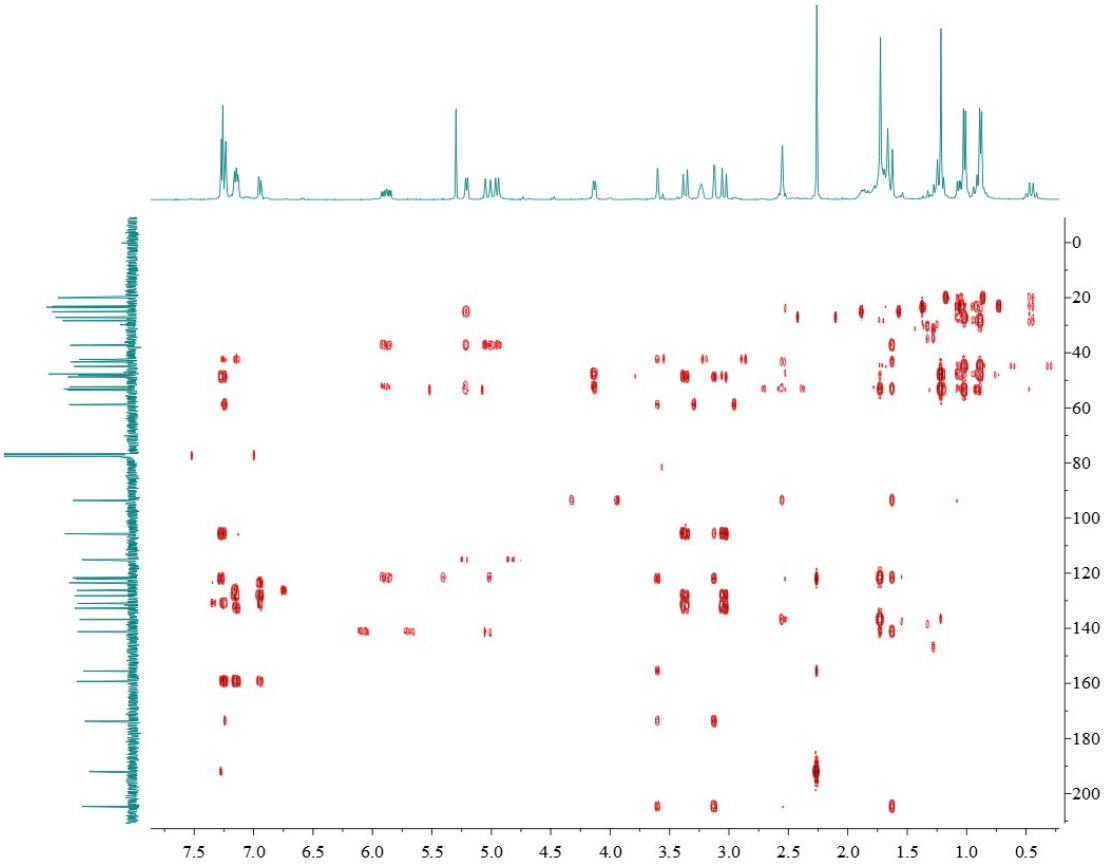


Fig. S8 NOESY spectrum of **1** in $CDCl_3$

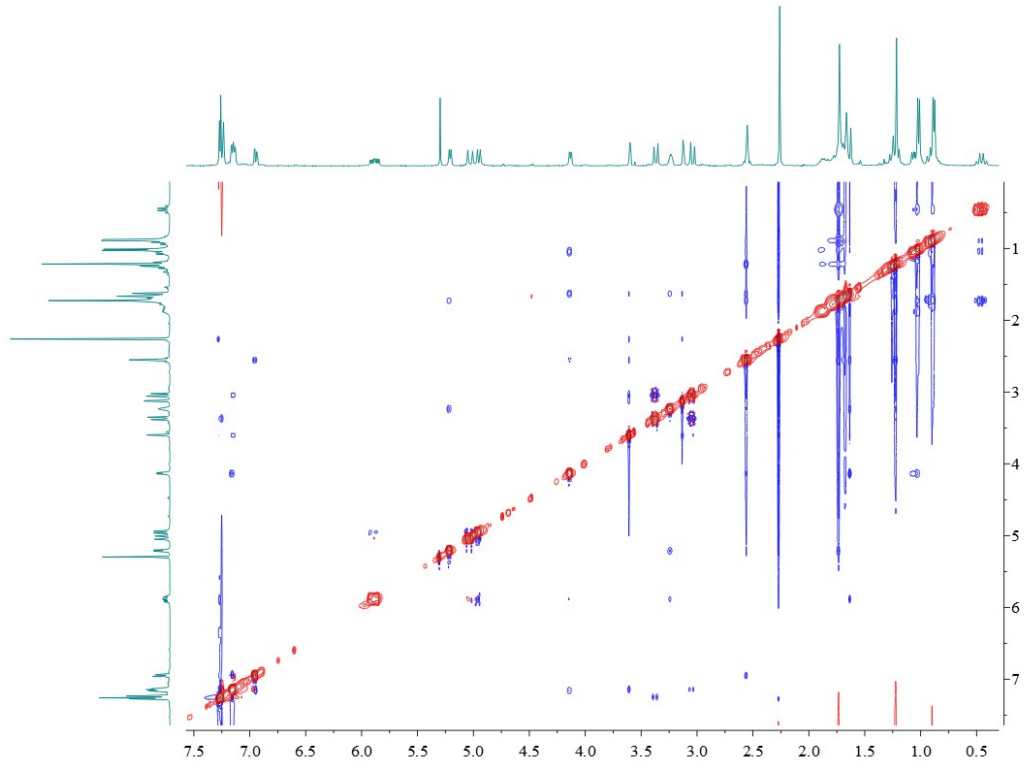
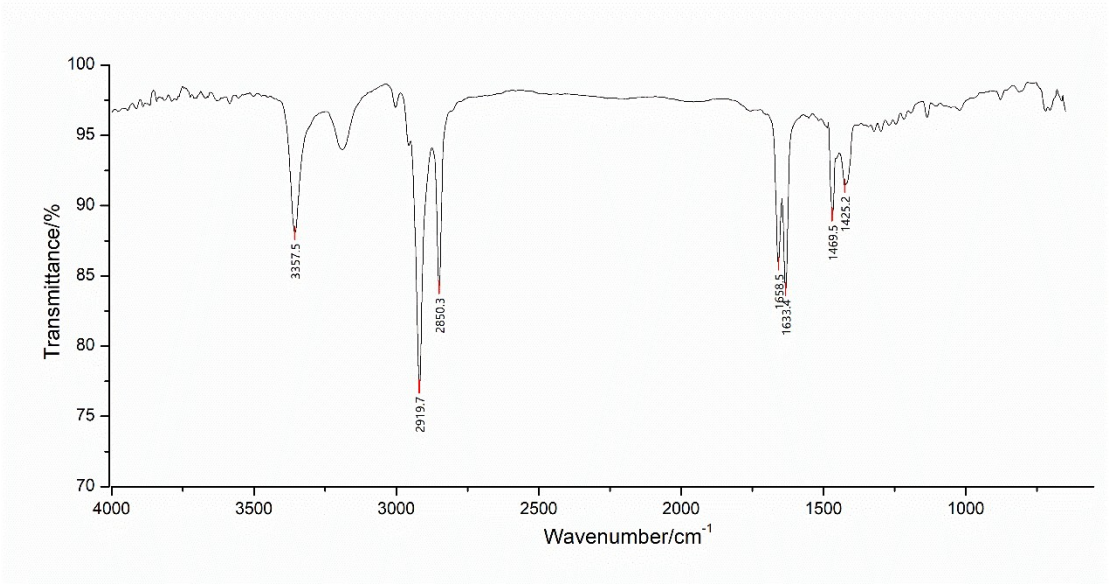


Fig. S9 IR spectrum of **1**



Experimental Section

1 General Experimental Procedures

Optical rotations were measured on a MCP 300 (Anton Paar) polarimeter at 28 °C. UV data was obtained on a PERSEE TU-1900 spectrophotometer. ECD data were obtained on a Chirascan™ CD spectrometer (Applied Photophysics). A Fourier transformation infra-red spectrometer coupled with infra-red microscope EQUINOX 55 (Bruker) was used to record the IR spectra. NMR spectra were recorded on Bruker Avance 400 spectrometer (^{1}H 400 MHz, ^{13}C 100 MHz). All chemical shifts (δ) were given in ppm with reference to the solvent signal (δ_C 77.1/δ_H 7.26 for $CDCl_3$), and coupling constants (J) were given in Hz. HR-ESIMS 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 4729 used in this study was isolated from an ascidian *Styela plicata*, which was collected in the Bay of Dapeng, Shenzhen City, Guangdong, Province, China, in

April 2016. The fungus was obtained using the standard protocol for isolation. 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. MH050972. A BLAST search result showed that the sequence was the most similar (99%) to the sequence of *Trichobotrys effusa* (compared to JX156367.1 KJ630313.1). A voucher strain was deposited in School of Marine Sciences, Sun Yat-Sen University.

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 four weeks. Following incubation, the mycelia and solid rice media were extracted with EtOAc. The extract was evaporated under reduced pressure to yield 9g of residue. The residue was then divided into four fractions (Fr. 1–Fr. 4) by column chromatography on silica gel, eluting with a gradient of petroleum ether/EtOAc from 1:0 to 0:1. Fr. 2 (419 mg) was subsequently separated by Sephadex LH-20 CC eluting with CH_2Cl_2/MeOH (v/v, 1:1) to give subfraction Fr. 2.6, which was purified on silica gel (CH_2Cl_2/MeOH v/v, 99:1) to yield compound **1** (2.5 mg).

*Trichobamide A (**1**):* white powder; $[\alpha]_D^{20}$ −28.1 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε): 230 (3.78), 266 (3.74) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$): 260 (−35.9) nm; IR (neat) ν_{max} 3357.5, 2919.7, 2850.3, 1658.5, 1633.4, 1469.5, 1425.2 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 554.3; HR-ESIMS *m/z* 554.2905 (calcd for 554.2906 $[M + H]^+$).

4 X-ray Crystallographic Analysis.

Compound **1** was obtained as colorless crystals from $CHCl_3$ using vapor diffusion method. The single crystal X-ray diffraction data was obtained on a Rigaku Oxford Diffraction with Cu-Kα radiation (λ = 1.54178A). The structures were solved by direct methods (SHELXS-97 and Olex2-1.2) and refined using full-matrix least-squares

difference Fourier techniques. Hydrogen atoms bonded to carbons were placed on the geometrically ideal positions by the "ride on" method. Hydrogen atoms bonded to oxygen were located by the difference Fourier method and were included in the calculation of structure factors with isotropic temperature factors. Crystallographic data for **1** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: 44-(0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).

*Crystal data of (**1**):* $C_{35}H_{41}NO_5(CHCl_3)$, *Mr* = 675.06, orthorhombic, a = 15.04610(10) Å, *b* = 28.6830(2) Å, c = 7.87240(10) Å, α = 90.00, β = 90.00, γ = 90.00, *V* = 3397.47(5) Å^3, space group $P2_12_12$, *Z* = 4, *D*calcd = 1.447 mg/m^3, μ = 1.320 mm^{-1}, and *F*(000) = 1513.0. Crystal dimensions: 0.41 × 0.32 × 0.14 mm^3. Independent reflections: 6774 (R_{int} = 0.0542). The final R_1 values were 0.0424, ωR_2 = 0.1111 (I > 2σ(I)). The goodness of fit on F^2 was 1.045. Flack parameter value was 0.005(13). CCDC number: 1829569.

5 Bioactivity assay

5.1 Cell culture and reagents

The human glioma cell lines, U251 was kindly provided by Cell Bank of Chinese Academy of Sciences, SNB19 was purchased from ATCC (USA). Cells were cultured in Dulbecco's Modfied Eagle's Medium (DMEM, Gibco, Carlsbad, CA, USA), containing 10% fetal bovine serum (FBS), 100 IU/ml penicillin, 100 μg/ml streptomycin (all from Gibco, Carlsbad, CA, USA) in a cell incubator with 5% CO_2 at 37˚C.

5.2 Cell proliferation assay

Cell proliferation was analyzed by Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. Briefly, U251 and SNB19 were digested and seeded at 1x10^3 cells/well in 96-well plates and cultured in 100 μl medium overnight. The cells were treated by trichobamide A with gradient concentrations (0, 1, 2, 5, 10 μmol) for the indicated time (12h, 24h and 48h). At each indicated time point,

CCK-8 solution (10 µl/well) was added and then incubated at 37˚C for 2 h. The optical density (OD) at 450 nm was recorded by a microplate teader (Tecan Infinite 200 PRO; Salzburg, Austria). Each experiment was performed three times.

5.3 Cell apoptosis assay

U251 and SNB19 cells ($1.5x10^5$/well) were seeded in a 6-well plate and incubated for 12h, then cells were treated with 10 µmol Trichobamide A for 24 h. Cell apoptosis was detected by Annexin V-phycoerythrin (PE)/7-aminoactinomycin D (7-AAD) assay (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Briefly, cells ($1.0x10^6$) were digested into single-cell suspension and washed twice with cold phosphate-buffered saline (PBS). Then the cells were incubated in 100 µl binding buffer with 7-AAD and PE-conjugated anti-Annexin V antibody, and incubated for 15 min at room temperature (25˚C) in dark before analysis by flow cytometry (Beckman Coulter Inc., Brea, CA, USA).

5.4 Western blotting

Protein samples were achieved from 1, 2, 5 and 10 µmol trichobamide A treated cells and their controls. Cells were lysed in RIPA buffer (Beyotime Biotechnology, Shanghai, China), protein concentration of each sample were determined by bicinchoninic acid (BCA) protein assay kit (Beyotime Biotechnology), and protein samples were boiled for 10 minutes. Protein of each sample (30µg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto nitrocellulose membranes. Proterin-laden membranes blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature, and incubated with primary antibodies (anti-Bax, anti-Bcl-2, anti-P53, anti-caspase 3 cleaved, anti-caspase 9 cleaved and anti-GAPDH, all from Cell Signaling Technology, CST, Shanghai, China) at 4˚C overnight. The membranes were washed with Tris-buffered saline (TBS) and incubated with horseradish peroxidase conjugated secondary antibodies (CST). Protein expression was detected by enhanced chemiluminescence (Millipore) and the semi-quantitative analysis was performed by ImageJ software (National Institutes of Health, Bethesda, MD, USA). Each experiment was performed three times.