Supplementary Information

New preaustinoids from a marine-derived fungal strain *Penicillium sp.* SF-5497 and their inhibitory effects against PTP1B activity

Jin-Soo Park[1], Tran Hong Quang[2], Nguyen Thi Thanh Ngan[3], Jae Hak Sohn[4], and Hyuncheol Oh[1]

[1]College of Pharmacy, Wonkwang University, Iksan 54538, Republic of Korea; [2] Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; [3] Institute of Genome Research, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; [4] College of Medical and Life Sciences, Silla University, Busan 46958, Republic of Korea

Correspondence: Prof. Hyuncheol Oh, College of Pharmacy, Wonkwang University, Iksan 54538, Republic of Korea.

E-mail: hoh@wonkwang.ac.kr

List of Supplementary Information

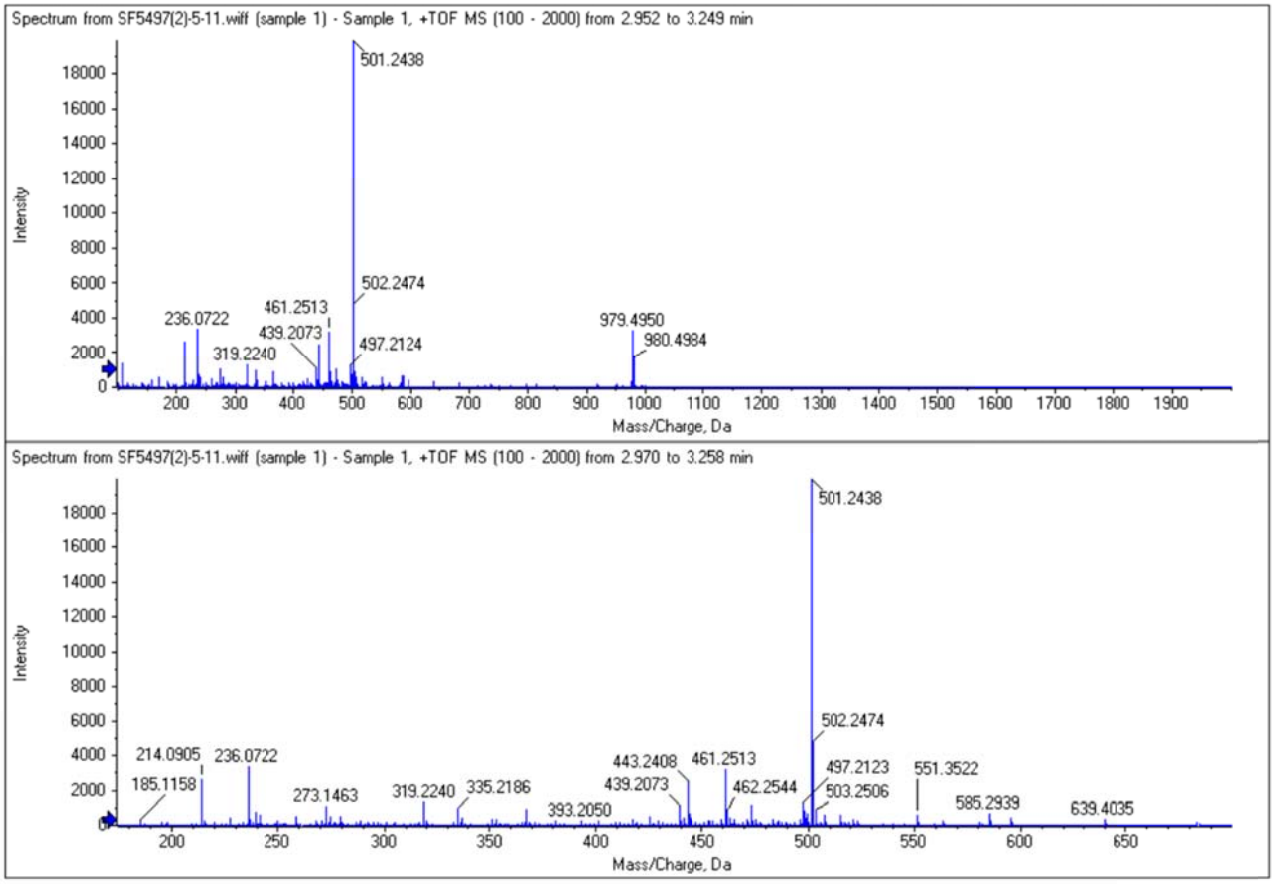


Figure S1. HRESIQTOF mass spectrum of 1

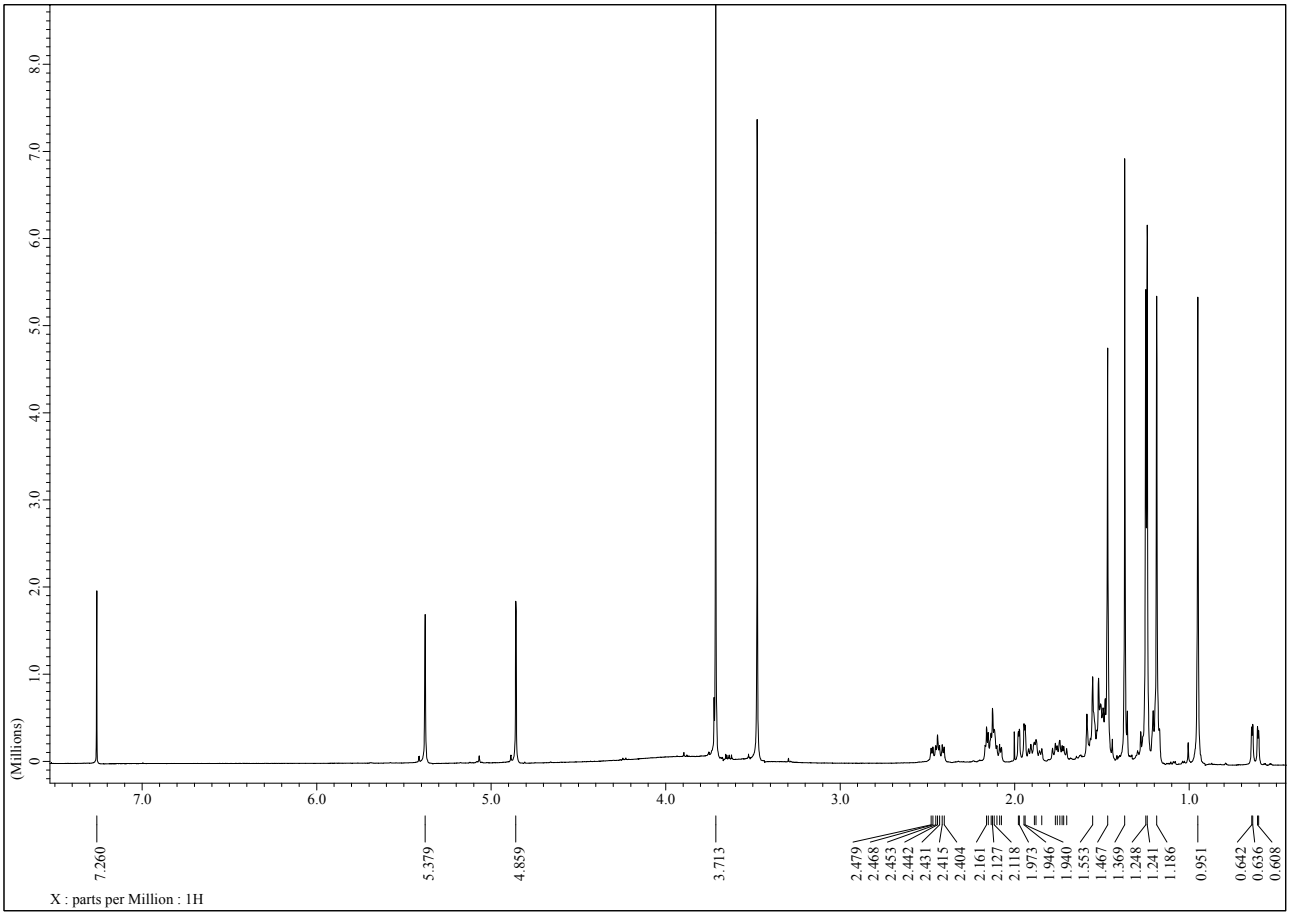


Figure S2. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound 1

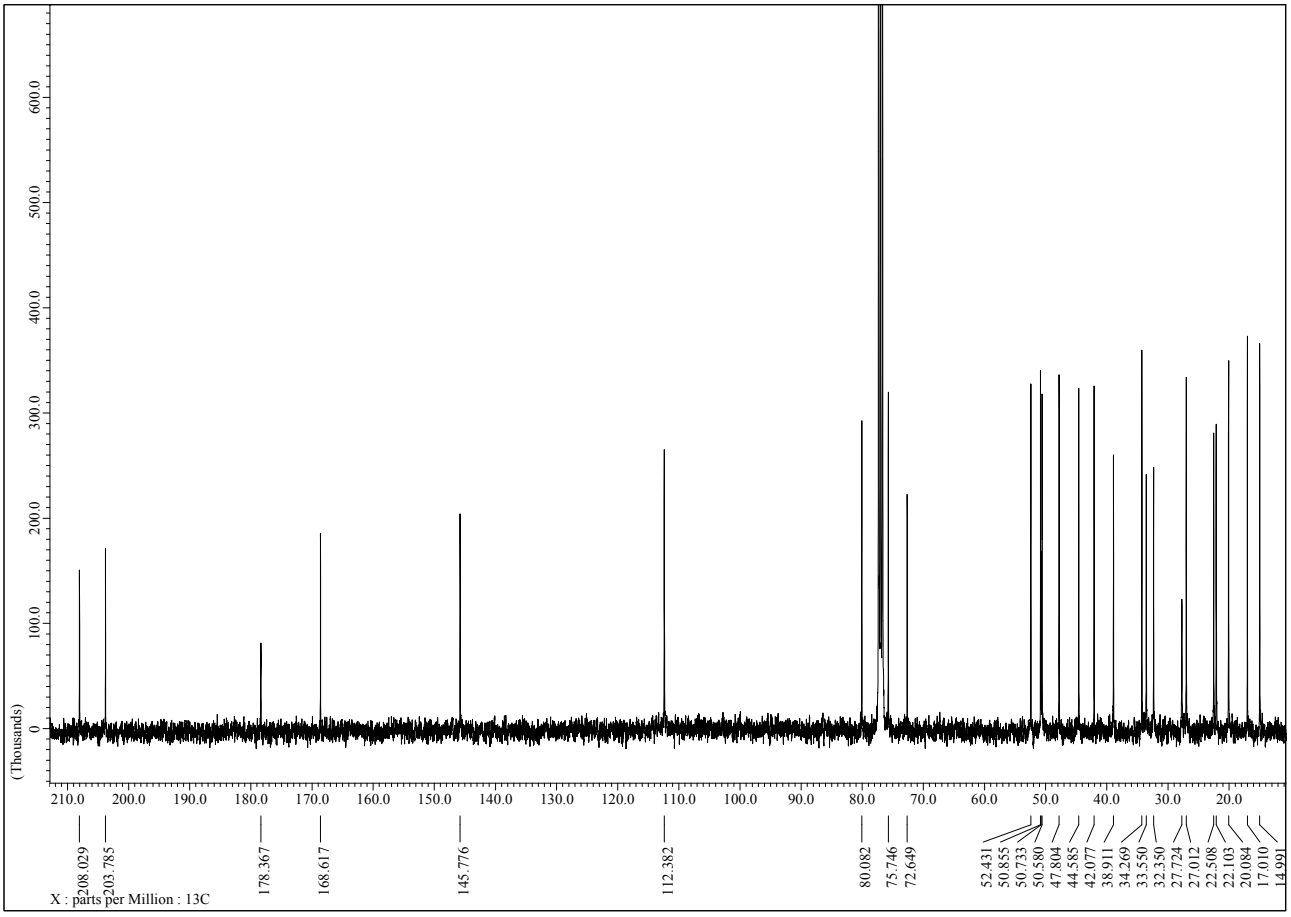


Figure S3. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound 1

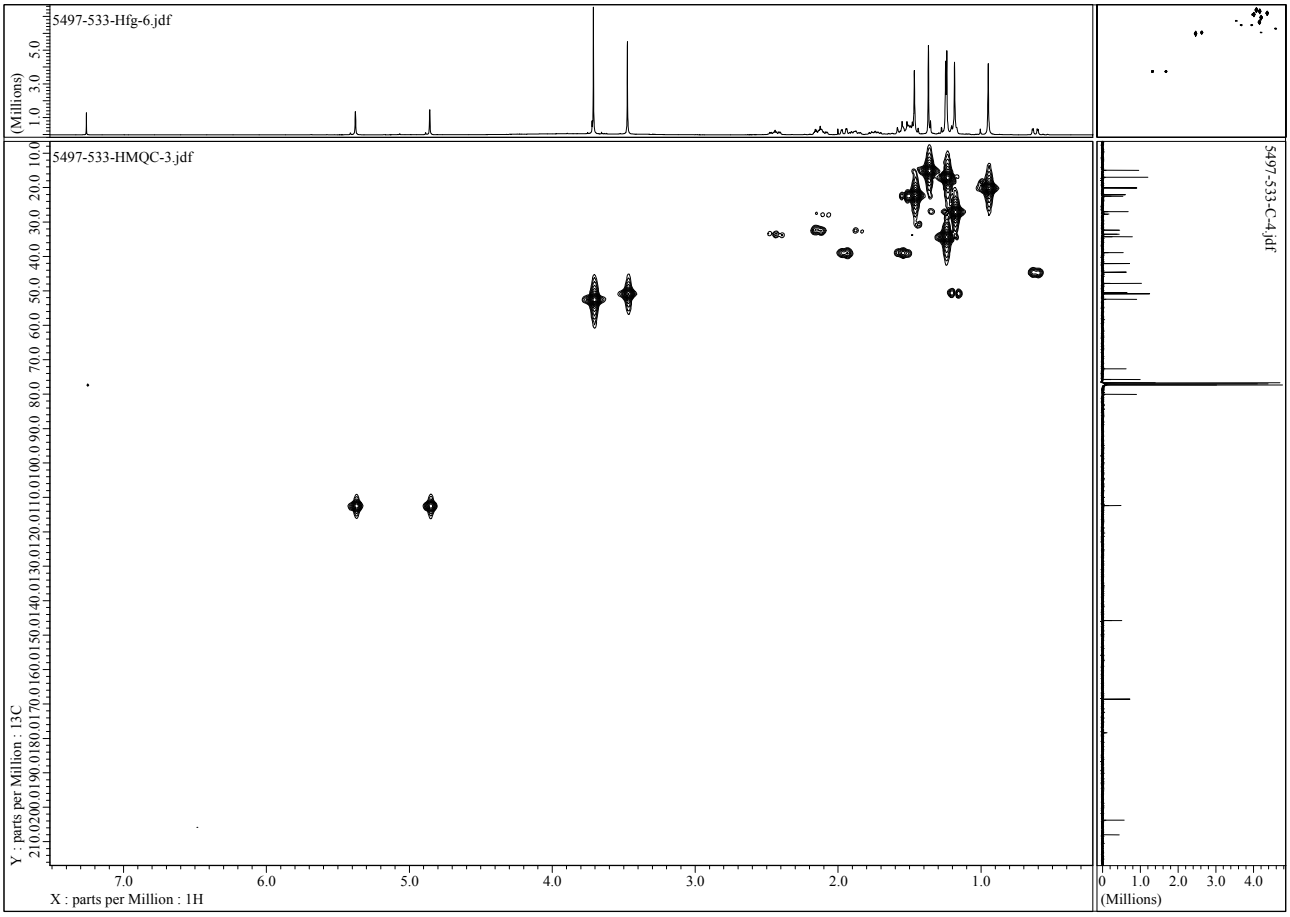


Figure S4. HMQC spectrum (400 MHz, $CDCl_3$) of compound 1

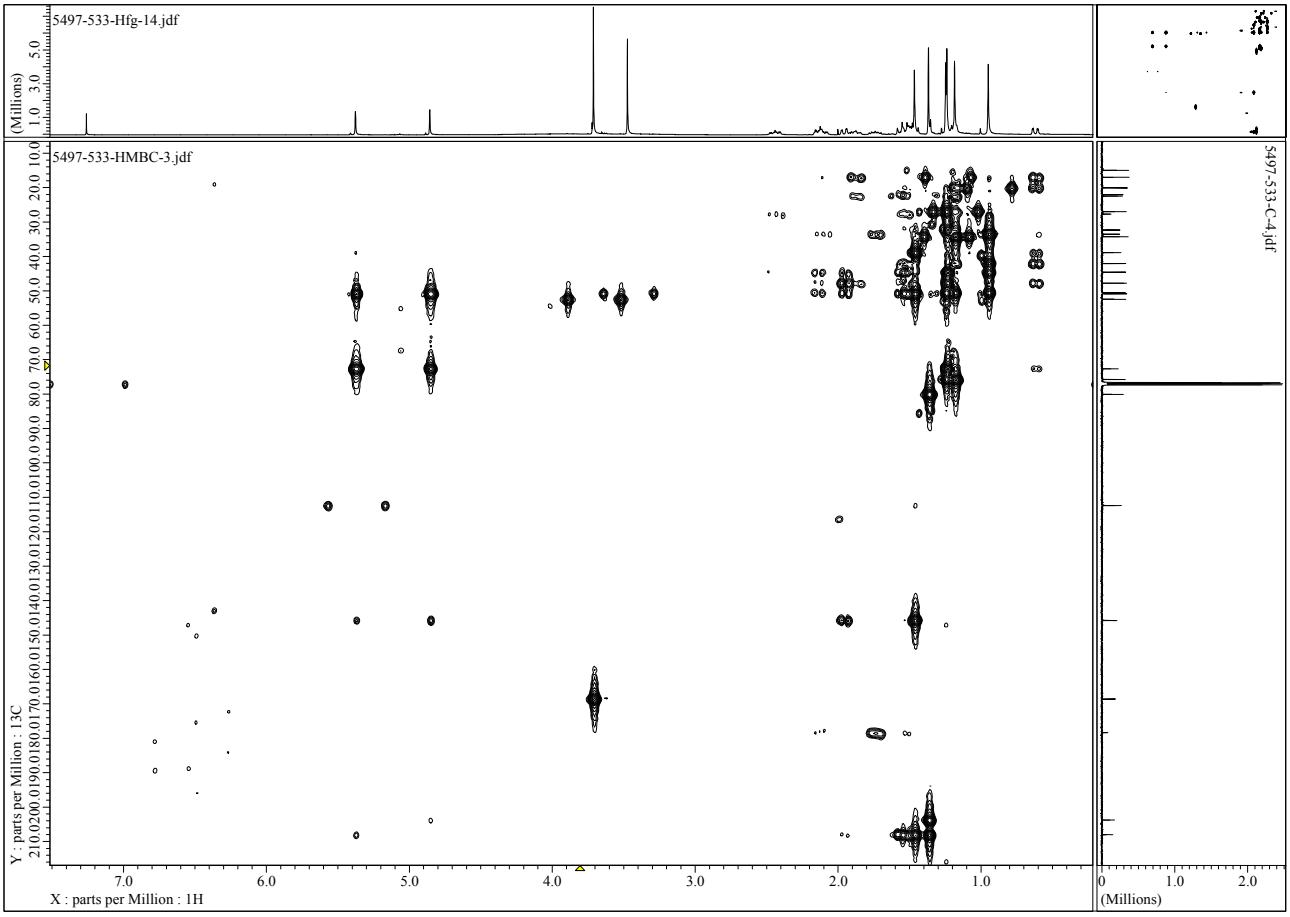


Figure S5. HMBC spectrum (400 MHz, $CDCl_3$) of compound 1

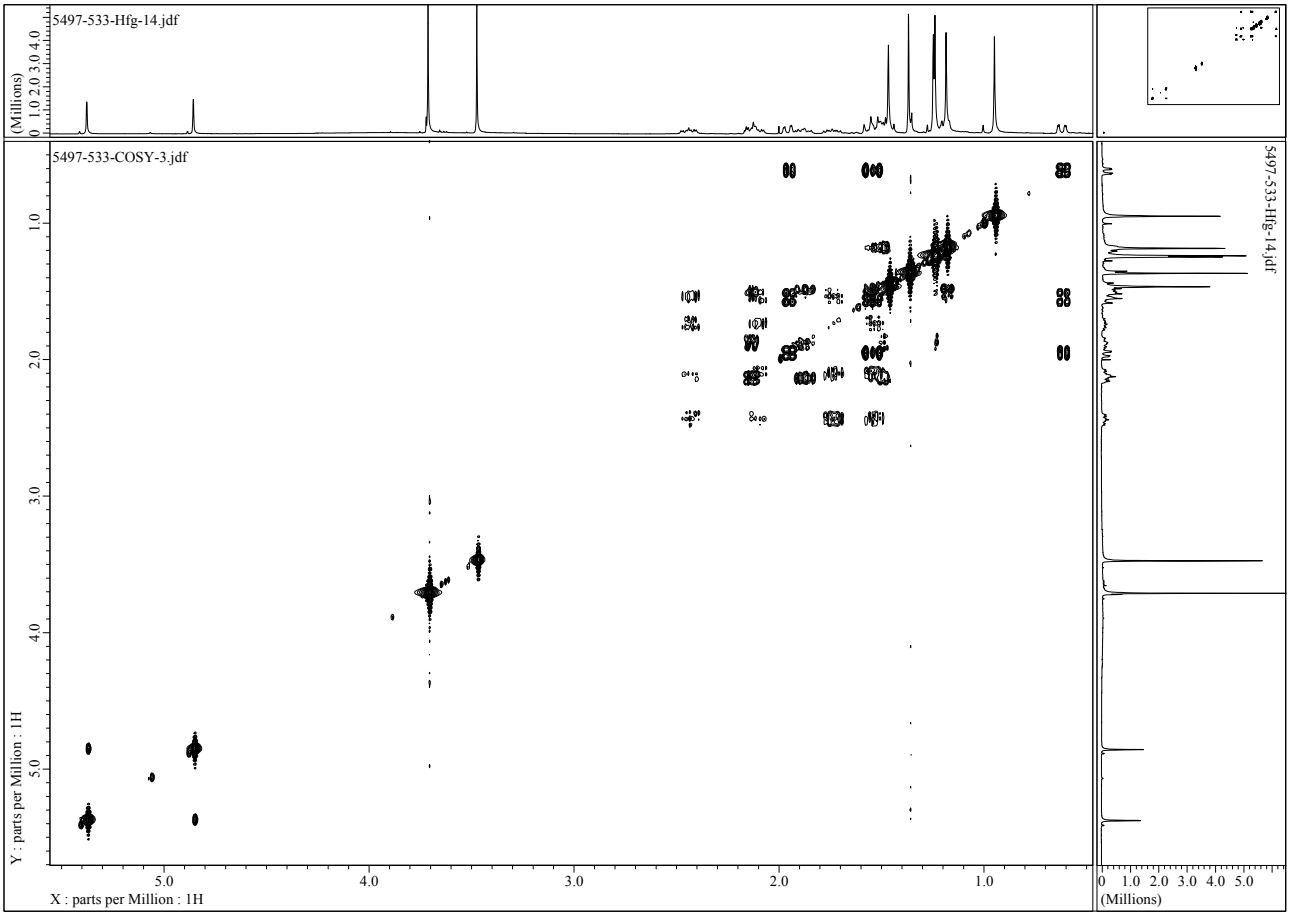


Figure S6. COSY spectrum (400 MHz, $CDCl_3$) of compound 1

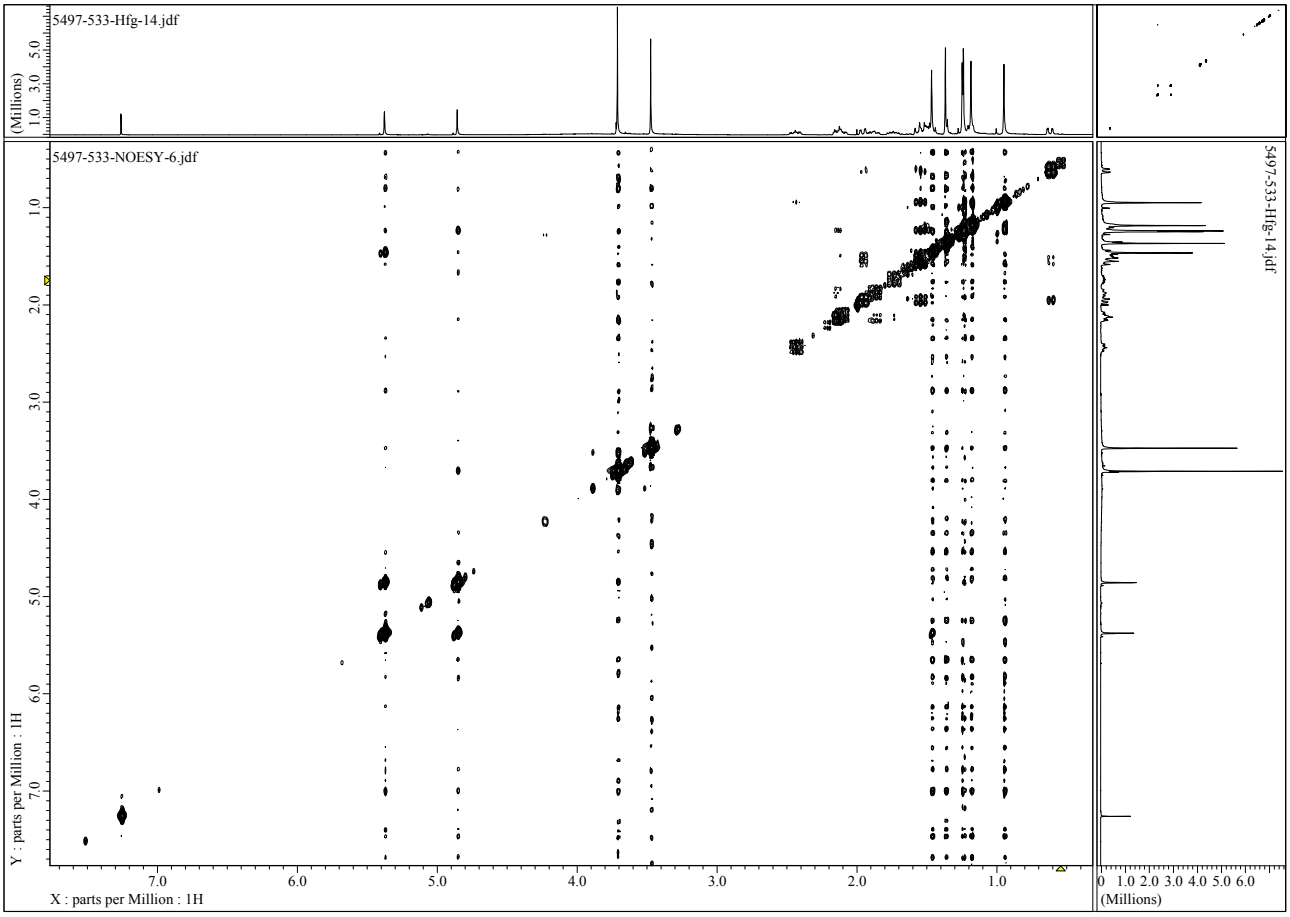


Figure S7. NOESY (400 MHz, $CDCl_3$) spectrum of 1

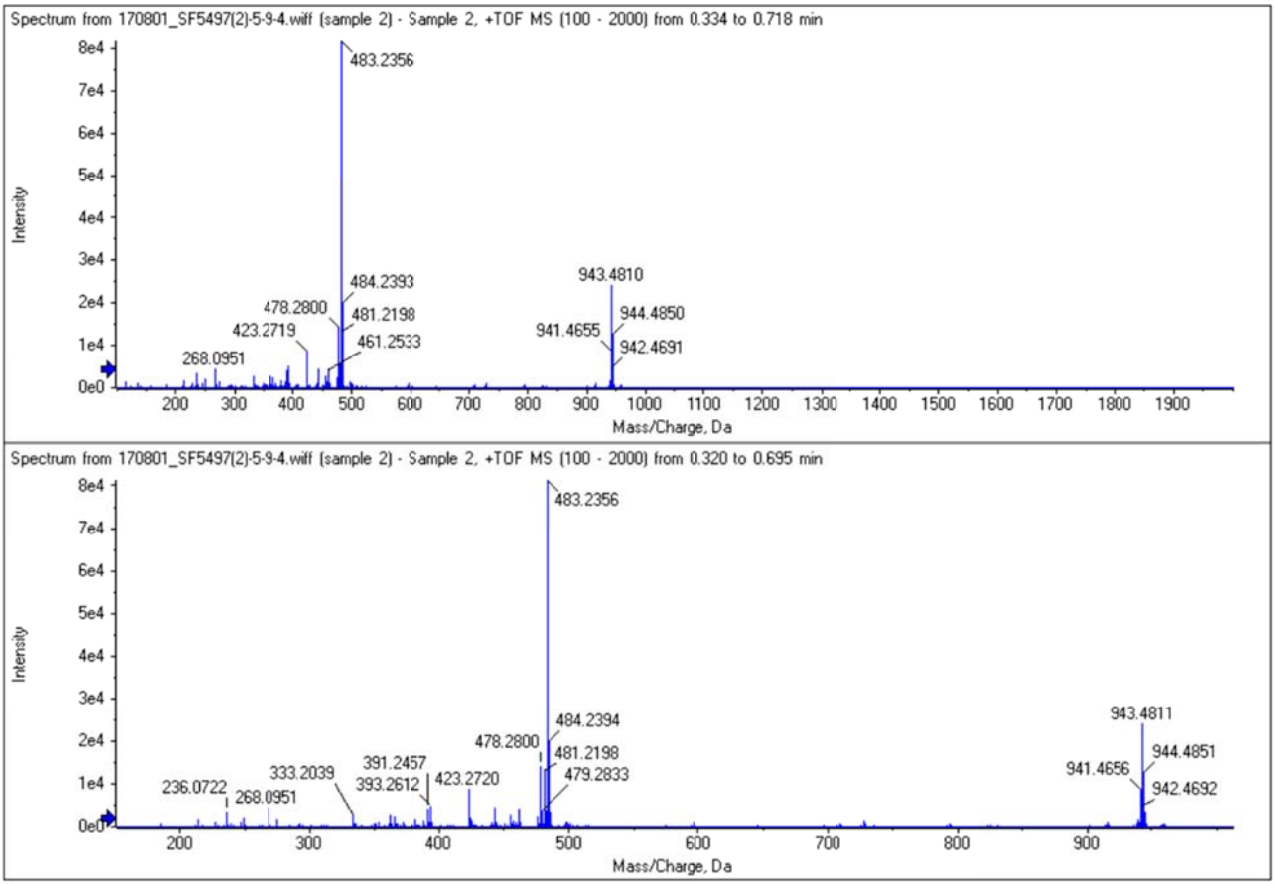


Figure S8. HRESIQTOF mass spectrum of compound 2

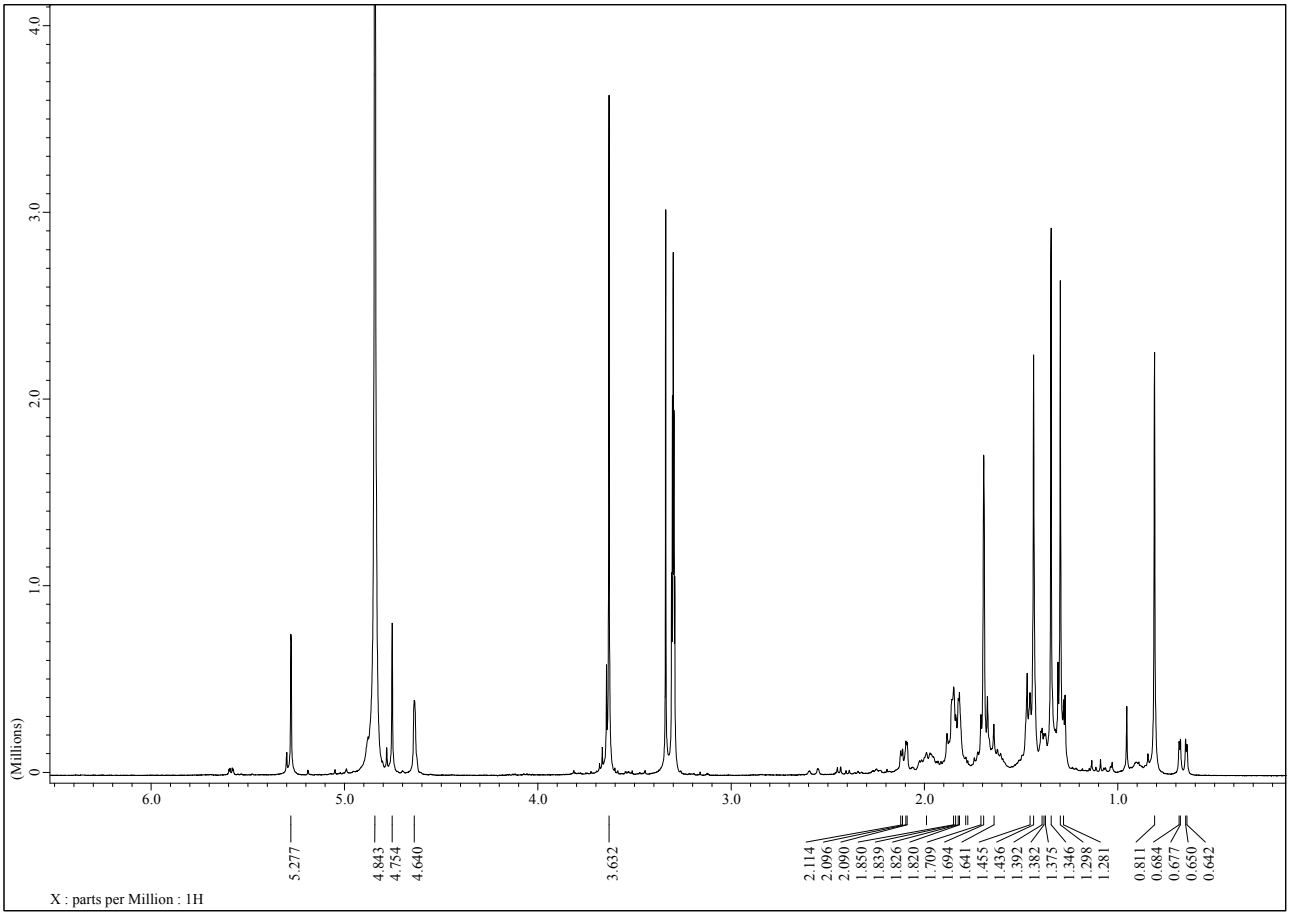


Figure S9. ^{1}H NMR spectrum (400 MHz, CD_3OD) of compound 2

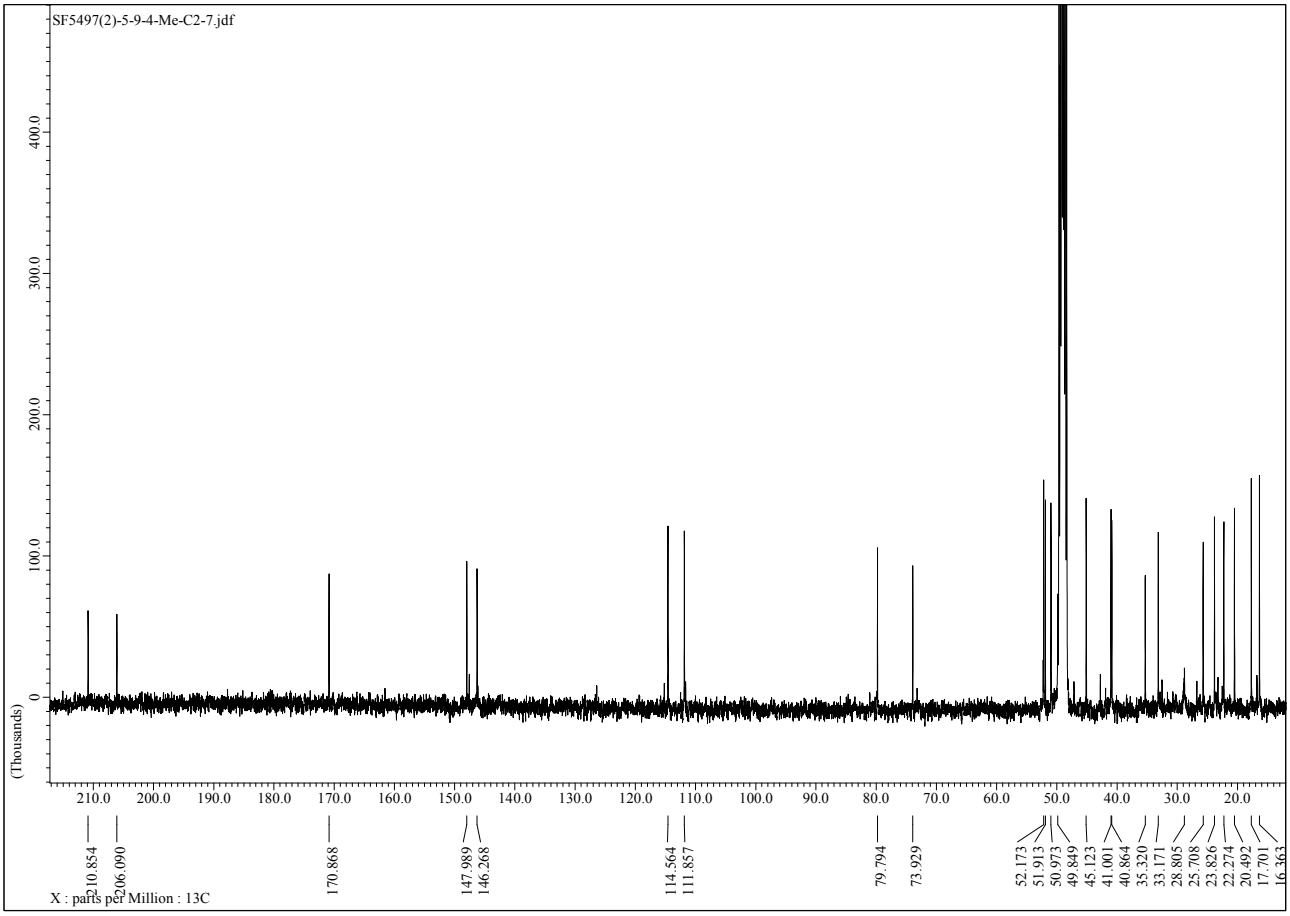


Figure S10. ^{13}C NMR spectrum (100 MHz, CD_3OD) of compound 2

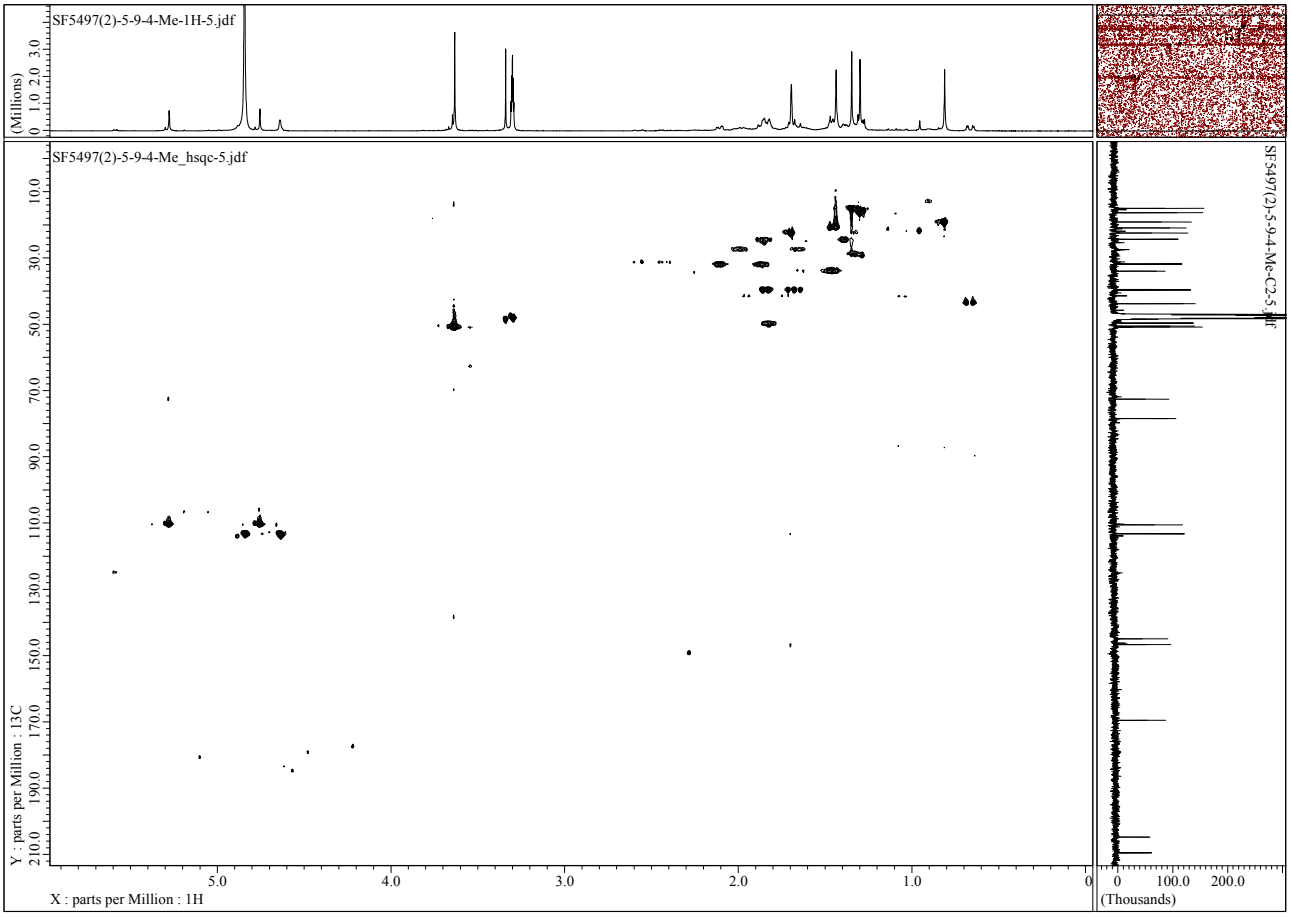


Figure S11. HSQC spectrum (400 MHz, CD_3OD) of compound 2

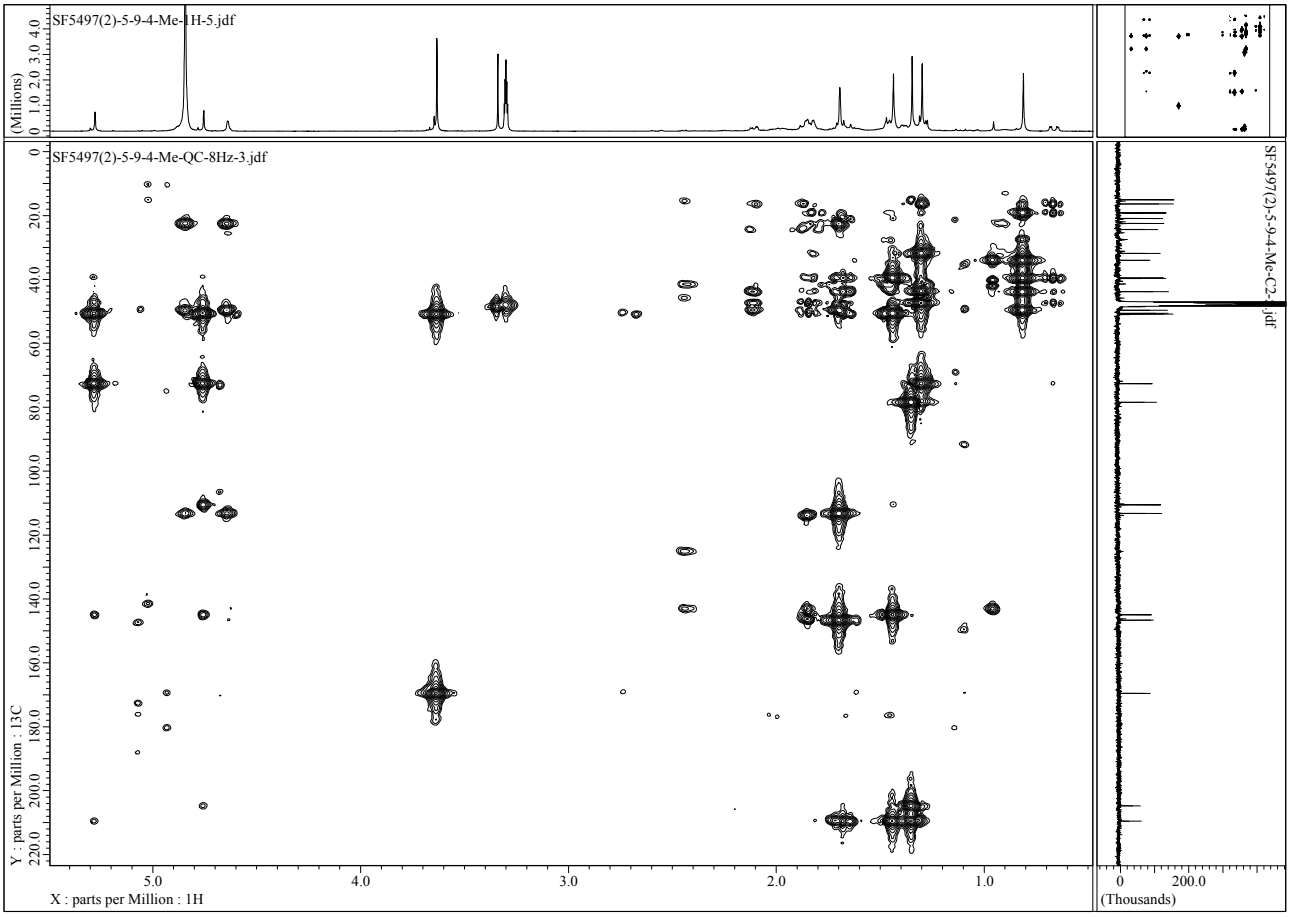


Figure S12. HMQC (J_{CH} = 8 Hz) spectrum (400 MHz, CD_3OD) of compound 2

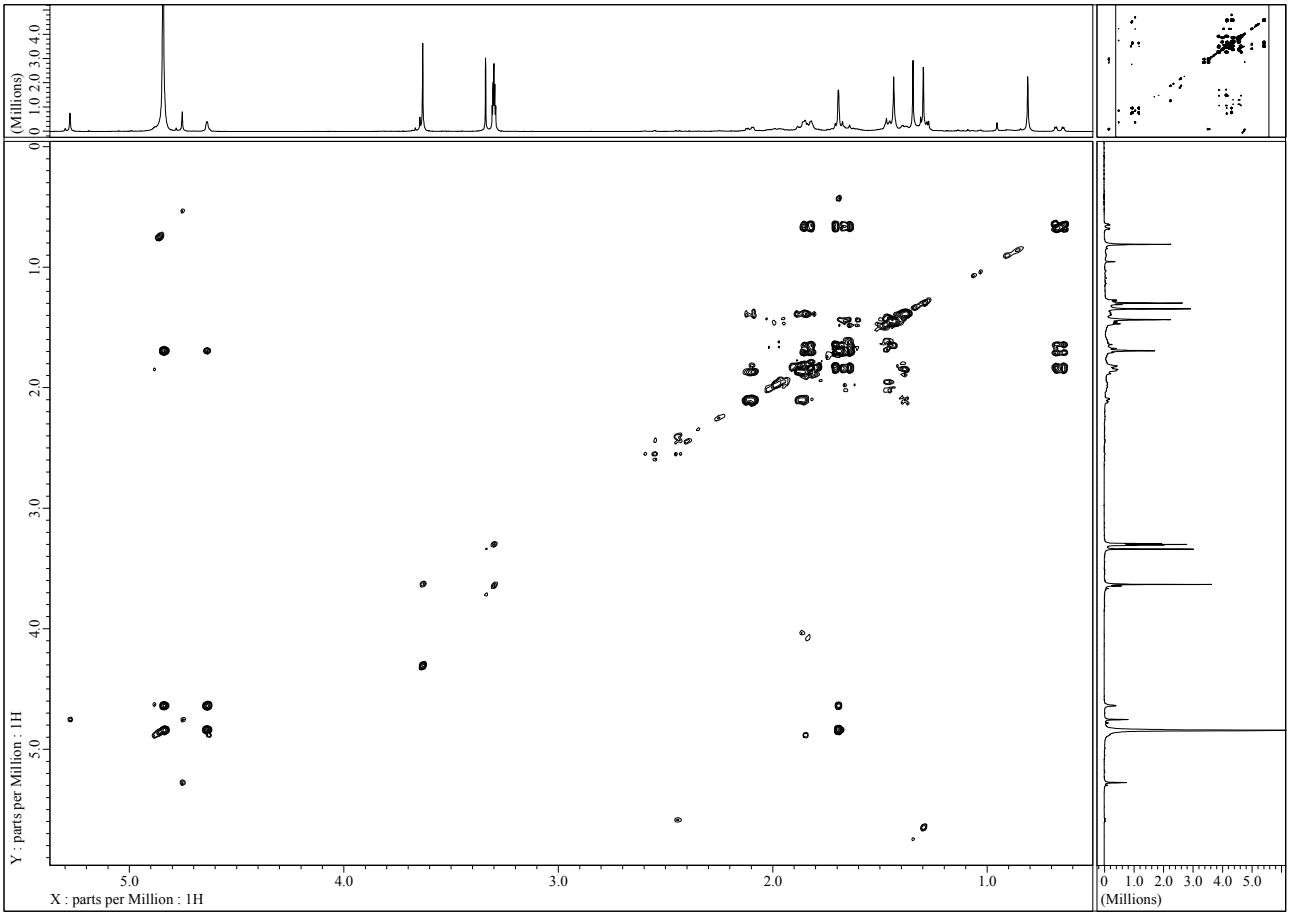


Figure S13. COSY spectrum (400 MHz, CD_3OD) of compound 2

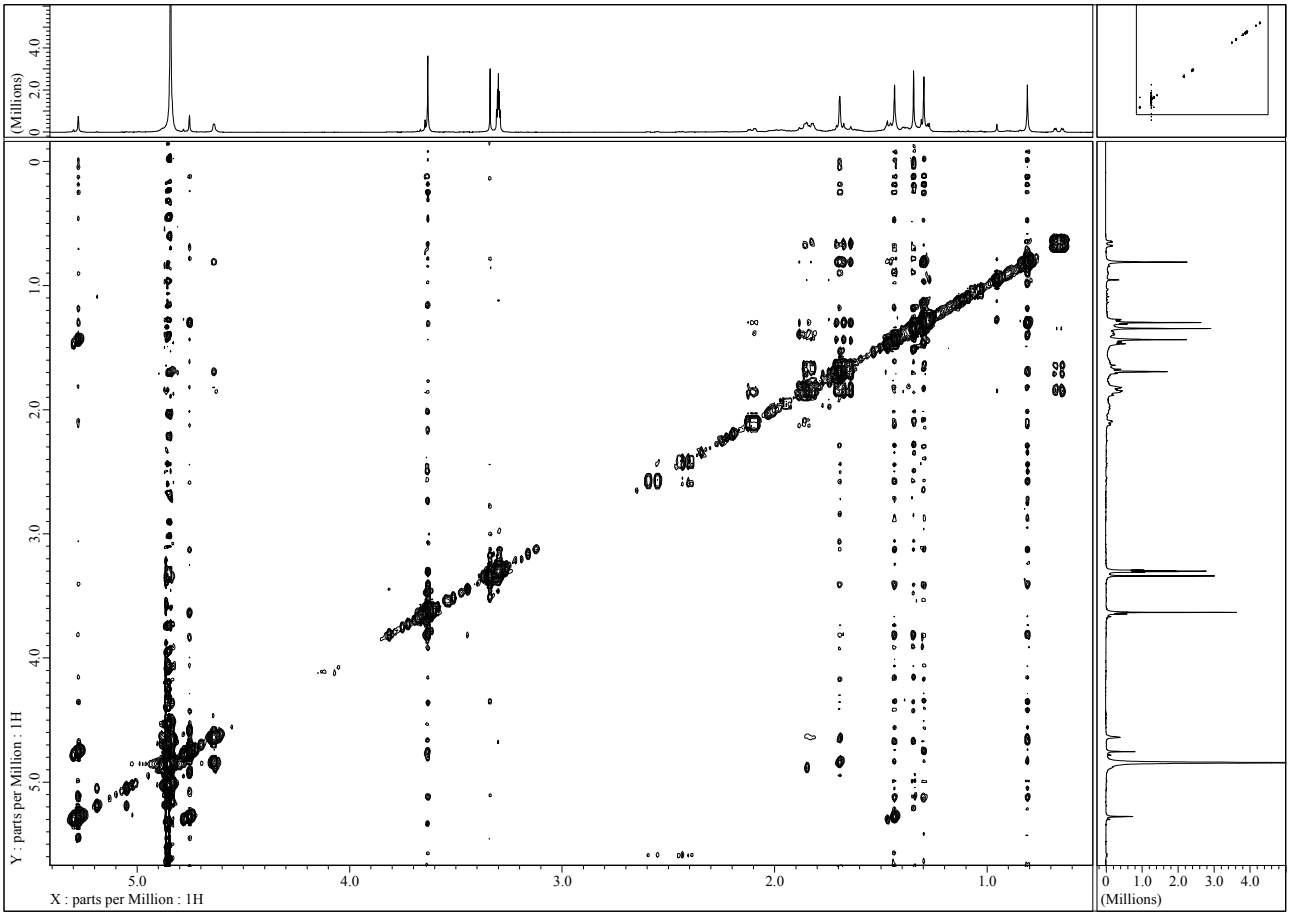
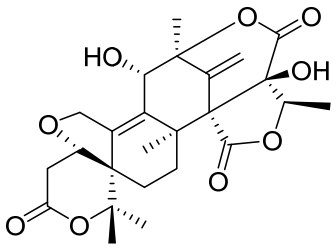
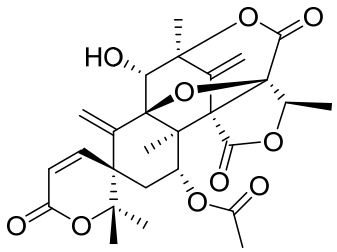


Figure S14. NOESY (400 MHz, $CDCl_3$) spectrum of 2



furanoaustinol



7-acetoxydehydroaustinol

Figure S15. Structures of previously isolated new meroterpenoid-type fungal metabolites furanoaustinol and 7-acetoxydehydroaustinol from *Penicillium* sp. SF-5497

MATERIALS AND METHODS

General Experimental Procedures

Optical rotations were recorded using a Jasco P-1020 polarimeter. NMR spectra (1D and 2D) were recorded in $CDCl_3$ using a JEOL JNM ECP-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) and chemical shifts were referenced relative to the corresponding residual solvents signals (δ_H 7.25/δ_C 77.0). HMQC, HSQC and HMBC experiments were optimized for $^1J_{CH}$ = 140 Hz, $^1J_{CH}$ = 140 Hz and $^nJ_{CH}$ = 8 Hz, respectively. HRESIMS data were obtained using an ESI Q-TOF MS/MS system (AB SCRIEX Triple). TLC was performed on Kieselgel 60 F_{254} (1.05715; Merck) or RP-18 F_{254s} (Merck) plates. Spots were visualized by spraying with 10 % aqueous H_2SO_4 solution, followed by heating. Column chromatography was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck) and YMC octadecyl-functionalized silica gel (C_{18}). HPLC separations were performed on a Synergi semiprep-C_{18} column (21.2 × 150 mm; 4 μm particle size; 80 Å pore size, 5 mL/min). Compounds were detected by UV absorption at 210 and 254 nm.

Fungal Material and Fermentation

Penicillium sp. SF-5497 was isolated from a sea- sand sample collected at Gijang-gun, Busan (N 35° 222257′ E 129° 239238′) on April 5, 2010. One gram of the sample was mixed with sterile seawater (10 mL). A portion (0.1 mL) of the filtered sample was processed utilizing the spread plate method in potato dextrose agar (PDA) medium containing seawater. The plate was incubated at 25 °C for 14 days. After subculturing the isolates several times, the final pure cultures were selected and preserved at -70 °C. The fungal strain SF-5497 was identified based on the analysis of the ribosomal RNA (rRNA) sequence. A GenBank search with the 28S rRNA gene of SF-5497 (GenBank accession number KU341382) indicated *Penicillium brasilianum* (HM469396), *P. simplicissimum* (HM469430), and *Trematosphaeria biappendiculata* (GU205229) as the closest maches showing sequence identities of 99.76%, 99.64% and 99.52%, respectively. Therefore, the marine-derived fungal strain SF-5497 was characterized as *Penicillium* sp., but could not be definitively identified to species.

Extraction and Isolation

The fungal strain SF-5497 was cultured on 45 Fernbach flasks each containing 200 mL of PDA with 3 % NaCl. The flasks were incubated at 25 ºC for 14 days. Extraction of the combined PDA media with EtOAc (1 L each) provided an organic phase, which was then concentrated *in vacuo* to yield a residue of SF5497(2) (7.2 g). The extract was subjected to reversed-phase (RP) C_{18} flash column chromatography (CC, 45 × 380 mm), and eluted with a stepwise gradient of 20, 40, 60, 80, and 100% (v/v) MeOH in H_2O (500 mL each) to give fractions, SF5497(2)-1–6, respectively. Fraction SF5497(2)-5 was subjected to silica gel CC, and eluted with a gradient of CH_3Cl-MeOH (70:1-1:1) to give **1** and eleven fractions, SF5497(2)-5-1–10 and SF5497(2)-5-12. Fraction SF5497(2)-5-2 and SF5497(2)-5-3 were separated by semi-preparative RP HPLC, using a gradient elution of 50–85 % ACN in H_2O (containing 0.1 % formic acid) over 50 min to give **4** (1.7 mg, t_R = 23.4 min). Fraction

SF5497(2)-5-5 was separated by semi-preparative RP HPLC, using a gradient elution of 50–100 % MeOH in H_2O (containing 0.1 % formic acid) over 50 min to give **5** (1.6 mg, t_R = 29.7 min). Fraction SF5497(2)-5-9 was separated by semi-preparative RP HPLC, using a gradient elution of 70–100 % MeOH in H_2O (containing 0.1 % formic acid) over 50 min to give **2** (7.8 mg, t_R = 24.2 min). Fraction SF5497(2)-5-10 was separated by semi-preparative RP HPLC, using a gradient elution of 60–70 % MeOH in H_2O (containing 0.1 % formic acid) over x min to give **3** (3.1 mg, t_R = 40.2 min).

*Preaustinoid A6 (**1**)*: Colorless gum; $[\alpha]_D^{24}$ = –13.0 (c = 0.12 CH_3OH); UV ($CHCl_3$) λ_{max} (log ε) 240 (2.98); IR (KBr) ν_{max} 3432, 2934, 2816, 2780, 1600, 1354, 1249, 1042, 767, 611 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C NM R data ($CDCl_3$, 100 MHz), see Table 1; HRESITOFMS *m/z*: 501.2438 $[M+Na]^+$ (calcd. for $C_{26}H_{37}O_8$, 501.2459).

*Preaustinoid A7 (**2**)*: Colorless gum; $[\alpha]_D^{24}$ = – 44.6 (c = 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 203 (4.23); ^1H (CD_3OD, 400 MHz) and ^{13}C NMR data (CD_3OD, 100 MHz), see Table 1; HRESITOFMS *m/z*: 483.2356 $[M+Na]^+$ (calcd. for $C_{26}H_{37}O_8$, 483.2353).

Methylation of Preaustinoid A6 (1)

To a hexane solution (0.2 mL) containing 2.0 M solution trimethylsilyldiazomethane ($TMSCHN_2$) was added a MeOH solution (0.5 mL) of **1** (1.5 mg). After stirring for 3 h at room temperature, the solution was concentrated under reduced pressure, and the residue was separated by Silica gel preparative TLC, using Hexane-EtOAc as eluents to give a methylated product, **1a** (1.0 mg).

*Compound **1a***: colorless gum; $[\alpha]_D^{23}$ = -29.2 (c = 0.1, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$) δ 5.38 (s, H-1′a), 4.86 (s, H-1′b), 3.71 (s, 8′-OCH_3), 3.65 (s, 3-OCH_3), 1.46 (s, H_3-9′), 1.37 (s, H_3-10′), 1.24 (s, H_3-15), 1.23 (s, H_3-12), 1.16 (s, H_3-14), 0.94 (s, H_3-13), 0.67 (dd, 3.2, 13.6, H-9); HRESIQTOFMS: *m/z* 515.2619 $[M+Na]^+$ (calcd for $C_{27}H_{40}NaO_8$, 515.2621);

PTP1B Inhibitory Activity Assay

PTP1B (human, recombinant) was purchased from ATGen Co., Ltd. The enzyme activity was measured in a reaction mixture containing 1 mM *p*-NPP in 50 mM Bis-Tris (pH 6.0), 2.0 mM EDTA, and 5.0 mM dithiothreitol (DTT). The reaction mixture was placed in a 37.5 °C for 30 min, followed by termination of the reaction with the addition of 10 N NaOH. The amount of *p*-NP produced was estimated by measuring the increase in absorbance at 405 nm. The non-enzymatic hydrolysis of 1.0 mM *p*-NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme. Inhibition kinetics studies were performed in the presence or absence of compounds **1**, with different concentrations of *p*-NPP. Data were fitted by nonlinear regression analysis according to a Michaelis-Menten kinetic model using Graphpad Prism 5.01.