Cycloheximide and Congeners, Inhibitors of Eukaryotic Protein Synthesis, from Endophytic Actinomycetes *Streptomyces* sps. YIM56132 and YIM56141[†]

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TITLE RUNNING HEAD: Cycloheximide and congeners from novel *Streptomyces* sps.

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[†]Dedicated to the late Professor C. Richard "Dick"/"Hutch" Hutchinson for his exceptional contributions to natural product biosynthesis, engineering, and drug discovery.

Supplementary Information

EXPERIMENTALS

General Experimental Procedures. Optical rotations were measured in DMSO on a Perkin-Elmer 241 polarimeter at the sodium D line (589 nm). ^{1}H and ^{13}C NMR spectra were recorded at 25 °C on a Varian Unity Inova 500 instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei. ^{1}H-^{1}H gCOSY (mixing time = 80 ms), gHMQC ($^{1}J_{CH}$ = 140 Hz), and *g*HMBC ($^{2\text{-}3}J_{XH}$ = 8.0 Hz) spectra were performed using standard VARIAN pulse sequences. Atmospheric pressure chemical impact-mass spectra (APCI-MS) were obtained on an Agilent 1100 HPLC–MSD VL quadrupole instrument. Electrospray ionization-mass (ESI-MS) spectra were acquired on an IonSpec HiResMALDI FT–Mass spectrometer with a 7 tesla superconducting magnet. Semipreparative HPLC was performed on a Varian liquid chromatograph system with an Altima-C18 column (5μ 10 × 250 mm). Column chromatography was performed either on silica gel (230–400 mesh, Natland International Corporation), Lichroprep RP-18 gel (40–63 μm, Merck, Dramstadt, Germany), or Sephadex LH-20 (Pharmacia).

Strain Isolation and Identification. The *Streptomyces* sps. YIM56132 and YIM56141 strains were isolated as endophytes from *Fagopyrum cumosum* and *Carex baccaus* in Yunnan province, of southwestern China. They were identified on the basis of polyphasic taxonomy. Phylogenetic analysis based on 16S rRNA gene sequences revealed low 16S rRNA similarity (< 98%) with other species of the genus *Streptomyces*. Strains YIM56132 and 56114 have been deposited at Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, P. R. China.

Fermentation and Isolation. The inoculum was prepared by introducing the periphery of 7-day-old petri dish cultures of *Streptomyces* sps. YIM56132 and YIM56141 into 250-mL flasks containing 50 mL of the ISP2 broth, followed by shaking (250 rpm) continuously for 2 days at 28.0 °C. The follow-up fermentation was accomplished by adding the inoculum 20 mL into 2-L flasks containing 400 mL (12 × 0.4 L) of the production culture broth [Yeast extract (2.0 g), malt extract (5.0 g), dextrose (2.0 g), in a final volume of 1.0 L H_2O, pH 7.0], and then shaking for 6 days under the same condition. Solid phase extraction of the broth using resin (XAD-16), filtration through cheesecloth, and elution of the resin with acetone afforded, after solvent removal under vacuum, two gummy extracts were obtained. Two extracts were subjected to the HPLC analysis and showed the similar HPLC trace. The YIM56141 extract was dissolved in 1-L water and extracted with EtOAc (800 mL) three times. The EtOAc extraction was dried using evaporator under vacuum and the extract (3.8 g) was generated. The extracts were then chromatographed on MPLC RP-18 gel column using H_2O/MeOH (5:95, 20:80, 50:50, 30:70, and 95:5, 1 L each) as the mobile phase to get six fractions A-F. Fractions C (50:50, 1.2 g) and D (90:10, 0.8 g) were further chromatographed over Sephadex LH-20 column eluted with pure MeOH to yield four fractions C1-C4 and D1-D4 for each fraction, respectively. These sub-fractions were further purified by semipreparative reverse phase HPLC and MPLC silica gel column eluted with $CHCl_3$ –MeOH. This afforded compounds **1** (7.5 mg), **2** (2.3 mg), **3** (5.2 mg), **4** (6.0 mg), **5** (2.5 mg), **9** (1.8 mg), **10** (2.6 mg), **11** (5.6 mg), and **12** (11 mg). Compounds **6** (9.7 mg), **7** (8.8 mg), and **8** (12.6 mg) were isolated from the strain YIM56132 following similar isolation procedures.

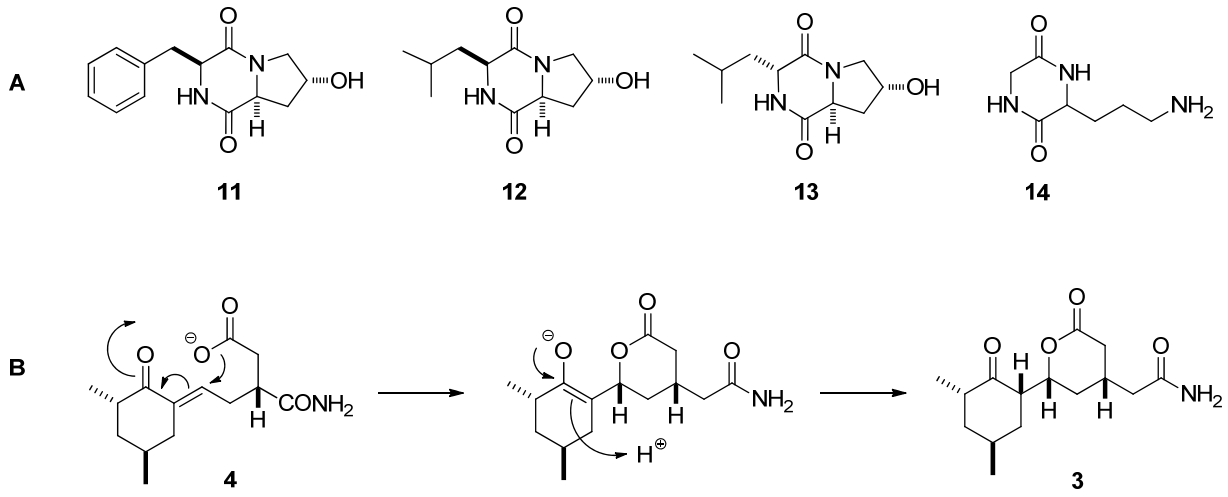
$NaBH_4$ reduction of 6 and 7 to afford 9 and 10. Compounds **6** (5 mg) and **7** (1.0 mg) were dissolved in 0.6 mL methanol with 1.0 mg and 0.3 mg $NaBH_4$, respectively. The mixers were stirred for 2 minutes and then the reaction was quenched by adding 0.2 mL acetone. Pure compounds **9** (4.5 mg) and **10** (0.9 mg) were obtained by evaporating the solvent and re-extracted with acetone.

Compound 4: $[\alpha]_D^{23}$ -7.1 (*c* 0.40, DMSO); APCI-MS (positive ion) *m*/*z* 282 (100); HR-ESI-MS (positive ion) *m*/*z* 282.17037 (calcd for $C_{15}H_{24}NO_4$ $[M+H]^+$, 282.16998) and *m*/*z* 304.15263 (calcd for $C_{15}H_{23}NO_4Na$ $[M+Na]^+$, 304.15193). ^{1}H and ^{13}C NMR data, see Table 1.

Compound 5: $[\alpha]_D^{23}$ +3.5 (*c* 0.15, DMSO); APCI-MS (positive ion) *m*/*z* 282 (100); HR-ESI-MS (positive ion) *m*/*z* 282.17019 (calcd for $C_{15}H_{24}NO_4$ $[M+H]^+$, 282.16998) and *m*/*z* 304.15274 (calcd for $C_{15}H_{23}NO_4Na$ $[M+Na]^+$, 304.15193). ^1H and ^{13}C NMR data, see Table 1.
Compound 9: APCI-MS (positive ion) *m*/*z* 278; ^1H NMR (500 Hz, DMSO-d_6): δ_H 2.34 (m, H-3a), 2.61 (m, H-3b), 2.24 (m, H-4), 2.30 (m, H-5a), 2.57 (m, H-5b), 1.51 (m, H-7a), 1.59 (m, H-7b), 4.91 (dd, *J* = 3.8, 9.0 Hz, H-8), 6.87 (d, *J* = 1.5 Hz, H-12), 6.76 (d, *J* = 1.5 Hz, H-14), 2.11 (s, H_3-15), 2.17 (s, H_3-16); ^{13}C NMR (125 Hz, DMSO-d_6): δ_c 174.1 (s, C-2), 38.8 (t, C-3), 27.8 (d, C-4), 37.7 (t, C-5), 174.0 (s, C-6), 43.5 (t, C-7), 67.2 (d, C-8), 128.1 (s, C-9), 150.3 (s, C-10), 132.0 (s, C-11), 124.9 (d, C-12), 130.1 (s, C-13), 125.0 (d, C-14), 16.9 (q, C-15), 21.0 (q, C-16).

Compound 10: APCI-MS (positive ion) *m*/*z* 294 (100); ^1H NMR (500 Hz, methanol-d_5): δ_H 2.47 (m, H-3a), 2.88 (m, H-3b), 2.62 (m, H-4), 2.41 (m, H-5a), 2.71 (m, H-5b), 1.85 (m, H-7a), 1.91 (m, H-7b), 4.85 (overlapped, H-8), 6.91 (br s, H-12), 6.78 (br s, H-14), 4.43 (s, H_2-15), 2.21 (s, H_3-16).

Figure S1. (**a**) Previously known diketopiperazines isolated from *Streptomyces* sp. YIM56141. (**b**) Proposed mechanism for silica gel-mediated conversion of **4** to **3** via intramolecular Michael addition.



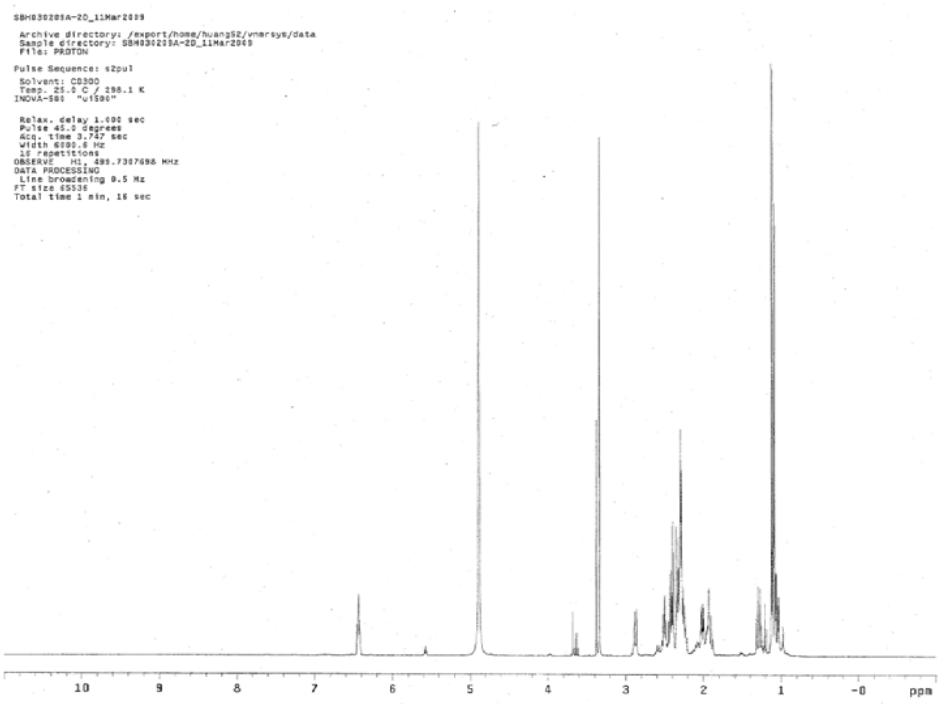


Figure S2. ^{1}H NMR spectrum of **4** in CD_3OD.

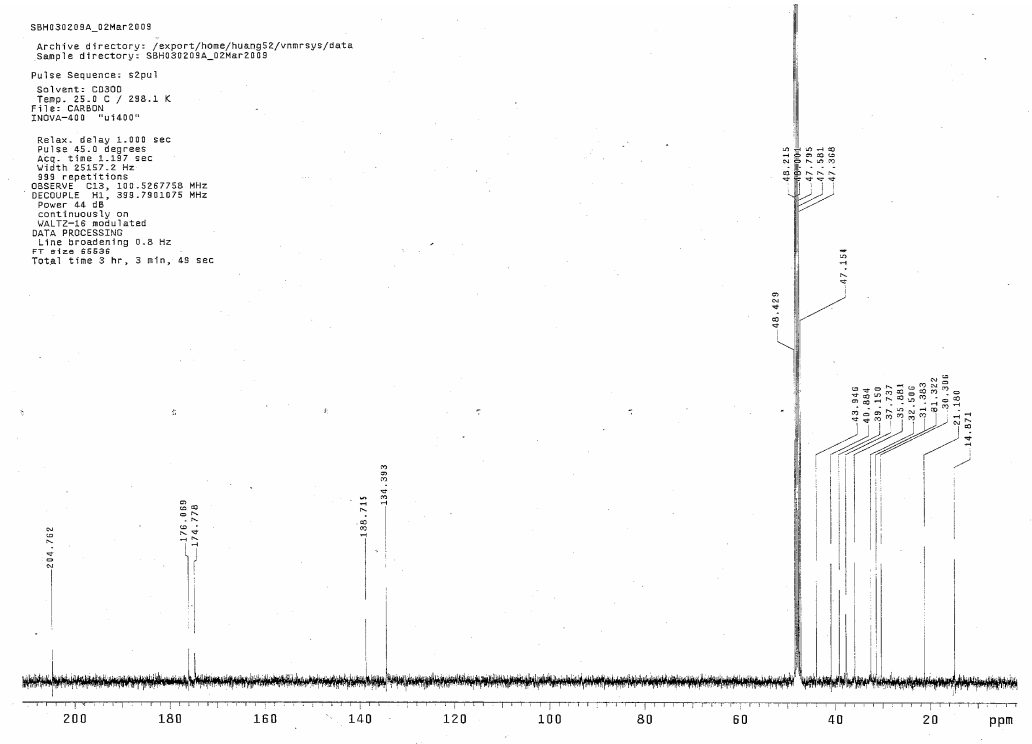


Figure S3. ^{13}C NMR spectrum of **4** in CD_3OD.

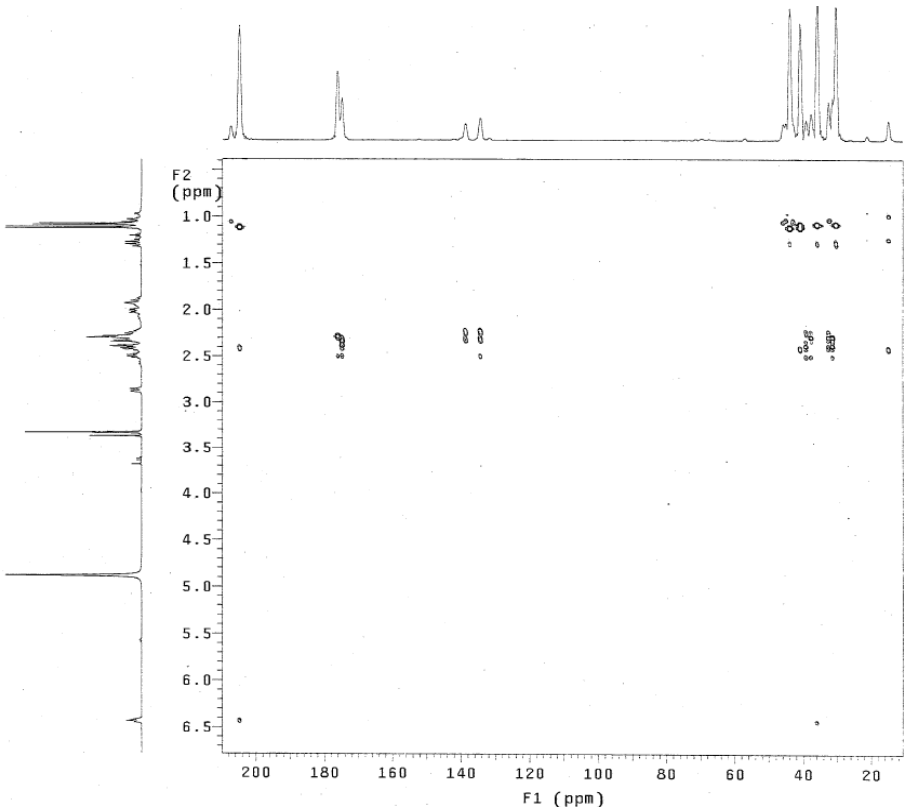


Figure S4. gHMBC spectrum of **4** in CD_3OD.

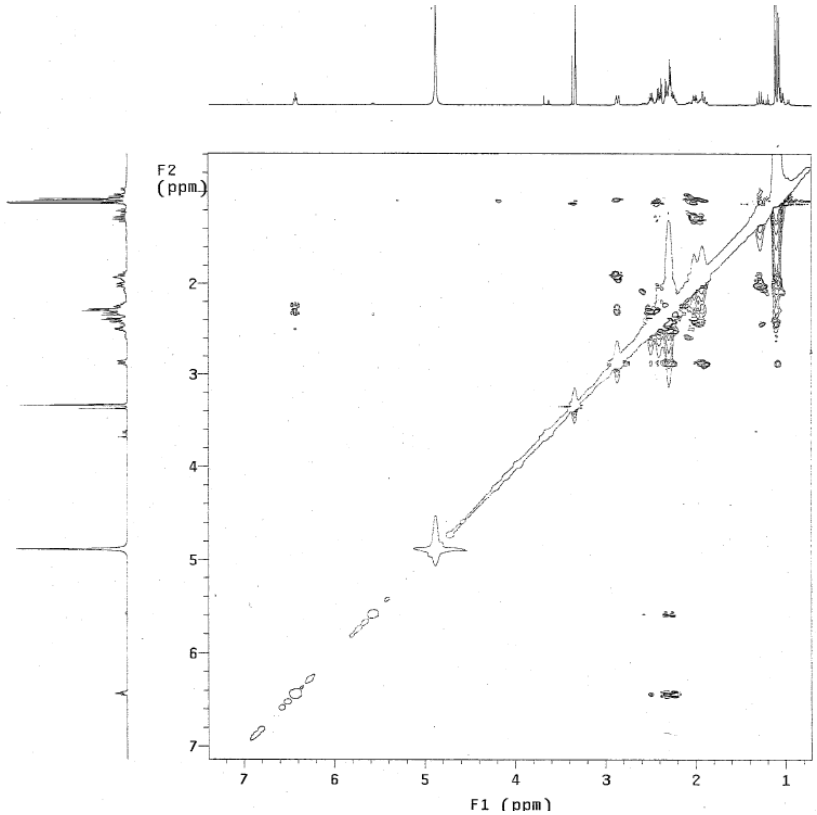


Figure S5. ROESY spectrum of **4** in CD_3OD.

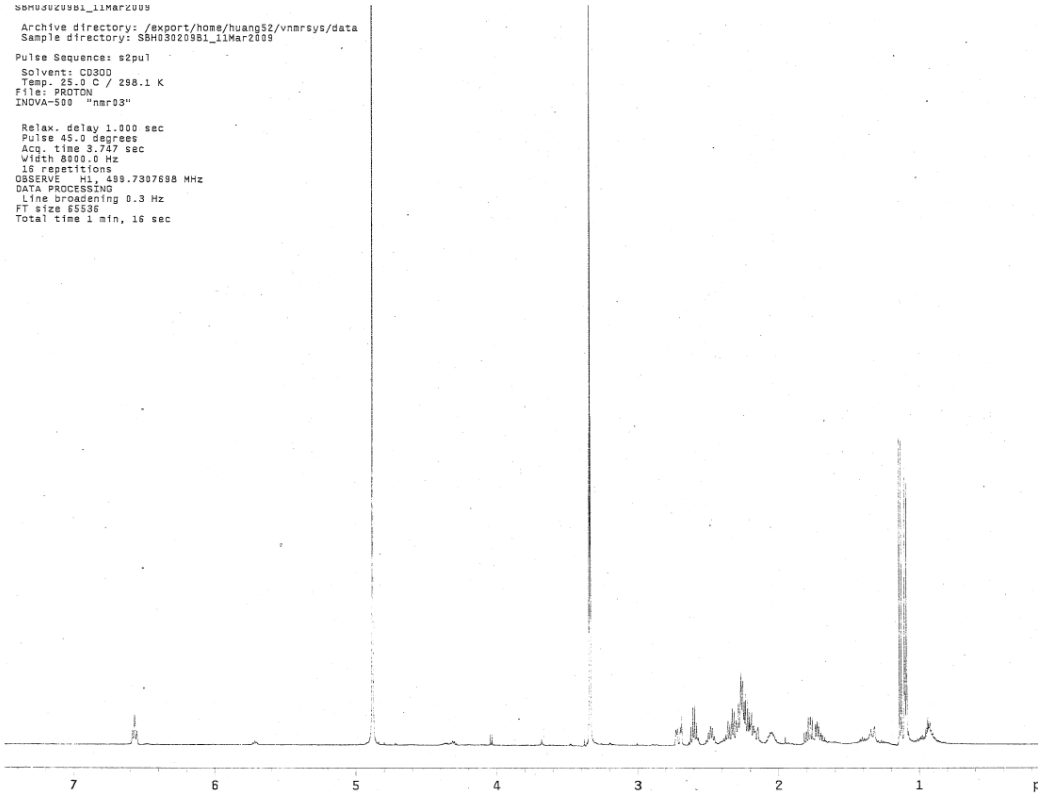


Figure S6. ^{1}H NMR spectrum of **5** in CD_3OD.

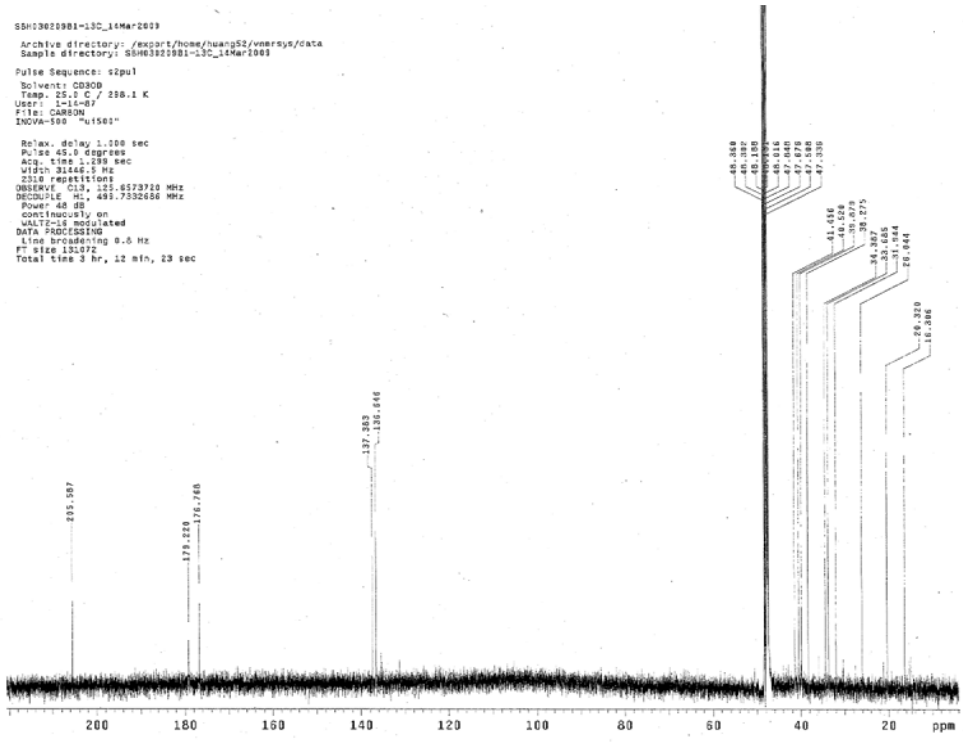


Figure S7. ^{13}C NMR spectrum of **5** in CD_3OD.

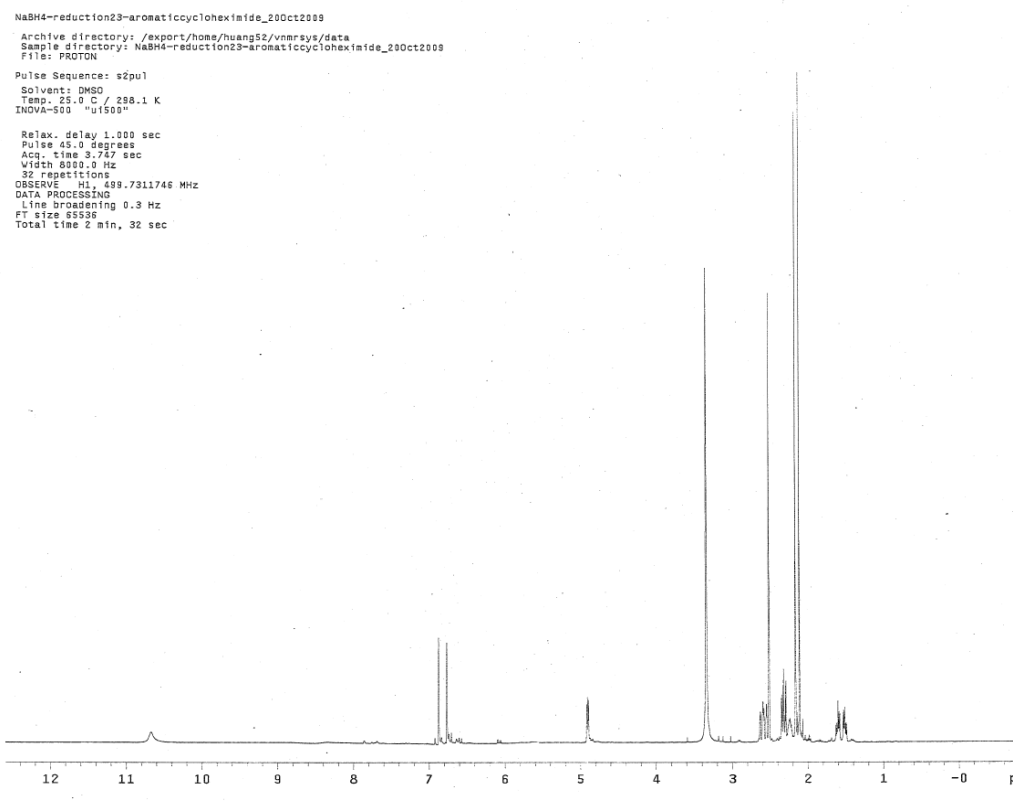


Figure S8. ^{1}H NMR spectrum of **9** in DMSO-d_6.

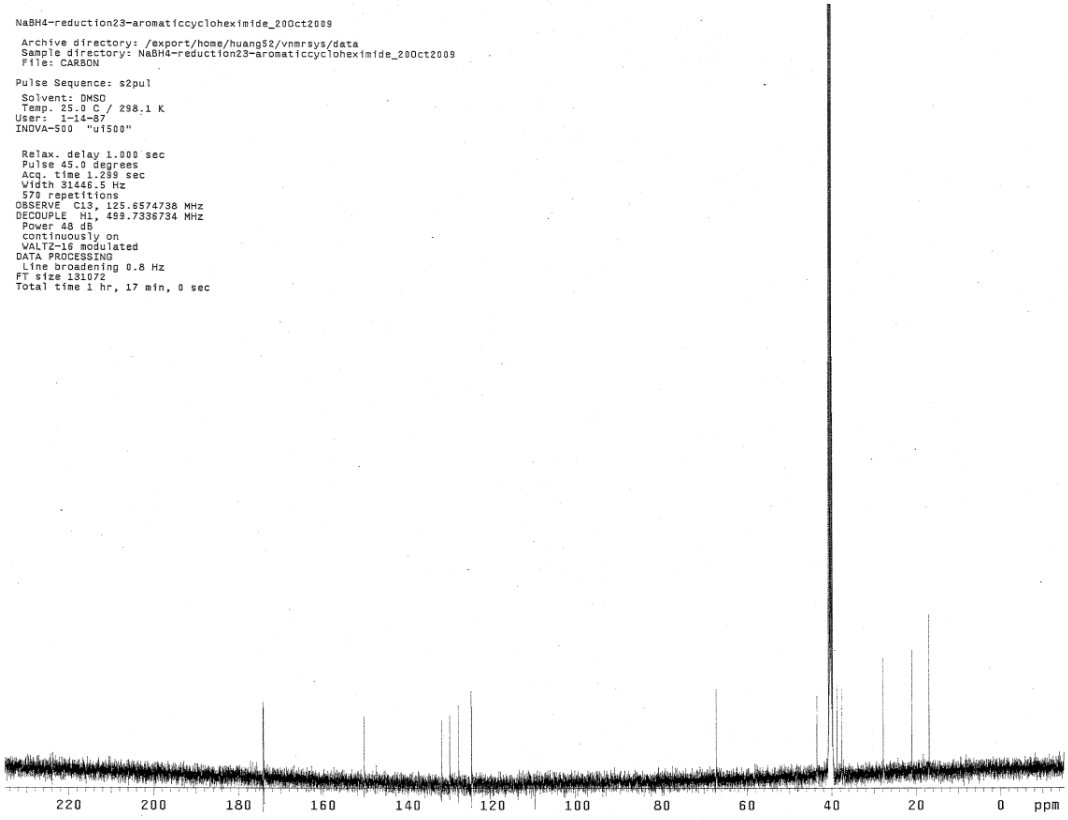


Figure S9. ^{13}C NMR spectrum of **9** in DMSO-d_6.

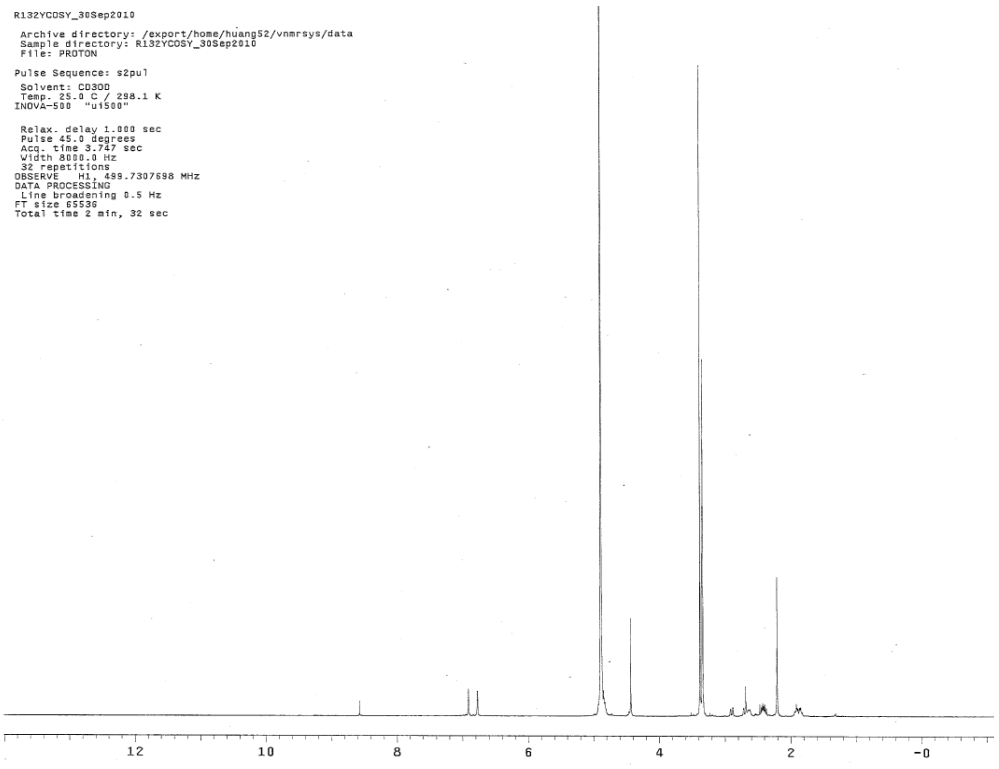


Figure S10. ^{1}H NMR spectrum of **10** in CD_3OD.

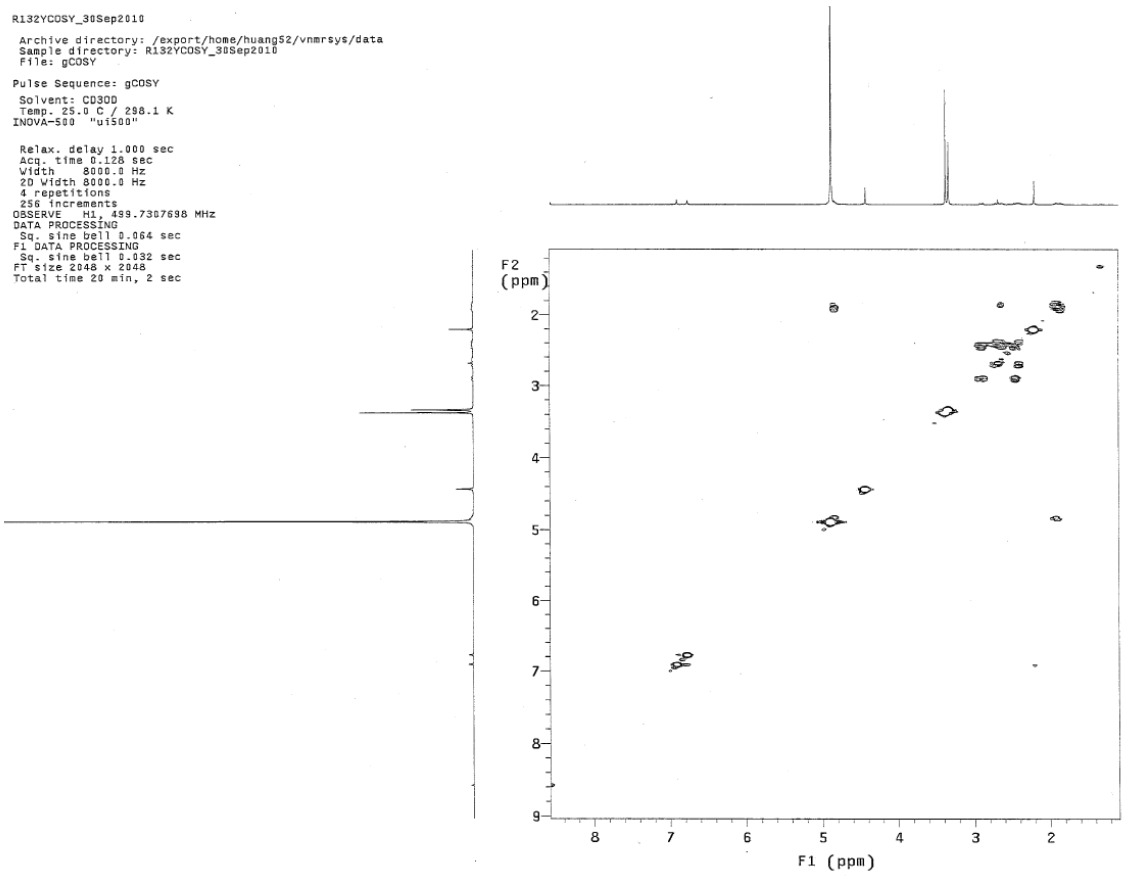


Figure S11. ^{1}H-^{1}H COSY NMR spectrum of **10** in CD_3OD.