SUPPLEMENTARY DATA

Terezine E, bioactive prenylated tryptophan analogue from an endophyte of *Centaurea stoebe*

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Abstract:

Fungal endophytes are considered promising sources of new bioactive natural products. In this study, a *Mucor* sp. has been isolated as an endophyte from the medicinal plant *Centaurea stoebe*. Through bioactivity-guided fractionation, the isolation of the new bioactive terezine E in addition to the previously reported 14-hydroxyterezine D was carried out. The isolated compounds were fully characterised by HRESIMS and 1D and 2D NMR analyses. Both compounds exhibited potent antiproliferative activity against K-562 and HUVEC cell lines and antifungal efficacy against the tested fungal strains.

Keywords: Terezine E, endophyte, cytotoxic, antimicrobial.

Content:

***General experimental procedures*.** NMR spectra were recorded on a Bruker DRX at 600 MHz for ^{1}H, and 150 MHz for ^{13}C NMR, respectively; chemical shifts are given in δ values (ppm). IR spectra were recorded on a Bruker FT-IR (IFS 55) spectrometer. UV spectra were recorded on a Cary 1 Bio UV–vis spectrophotometer (Variant). HPLC-MS measurements were recorded on an Agilent high performance 1100 series LC/MSD. HRESIMS were recorded on a Finnigan TSQ Quantum Ultra AM Thermo Electron. Open column chromatography was performed on silica gel 60 (Merck, 0.04–0.063 mm, 230–400 mesh ASTM) and Sephadex LH-20 (Pharmacia). TLC: silica gel plates (silica gel 60 F254 on aluminium foil or glass, Merck), spots were visualized by spraying with anisaldehyde/sulfuric acid, vanillin/sulphuric acid and Ehrlich`s reagent followed by heating. Preparative HPLC was performed on a Shimadzu HPLC system using a Nucleosil 100-5 C18 column (250 x 16 mm, pore diameter 100 Å) using a flow rate of 10 mL.min $^{-1}$ starting elution with 25% MeCN and ending with 100% MeCN in 45 min with a UV detector. All solvents used were spectral grade or distilled prior to use.

Antimicrobial assay. Quantitative determination of antifungal activity was performed by measurement of the minimal inhibitory concentration (MIC) according to the NCCLS guidelines using the broth micro dilution method (Wayne 2002). Briefly, 50 µL of the test compound solution in methanol were serially diluted by factor two with the culture medium. Then, the wells were inoculated with 50 µL of the test organism to give a final concentration of $6x10^3$ CFU mL^{-1}. After incubation of the microtiter plates at 37°C for 24 h, the MIC-values were read with a Nepheloscan Ascent 1.4 automatic plate reader (Lab systems, Vantaa, Finnland) as the lowest dilution of compound allowing no visible growth. The MIC for *Bacillus subtilis* was determined by the agar diffusion method (Bonev et al. 2008). Fifty µL of each of the 12 serial twofold dilutions were filled in agar (malt extract agar from Roth, Karlsruhe, Germany; seeded with 0.5 mL of a pretested mycelial solution) holes of 9 mm in diameter. After incubation for 24 h, the MIC was the lowest concentration giving an inhibition zone.

Antiproliferative and cytotoxic assays. These assays were performed as detailed before (Abdou et al. 2010). Briefly, cells of HUVEC (ATCC CRL-1730), K-562 (DSM ACC 10) and HeLa (DSM ACC 57) were cultured in DMEM (CAMBREX 12-614F), RPMI 1640 (CAMBREX 12-167F) and RPMI 1640 (CAMBREX 12-167F), respectively. All cells were grown in the appropriate cell culture medium supplemented with 10 $mL.L^{-1}$ ultraglutamine 1 (Cambrex 17-605E/U1), 500 $µl.L^{-1}$ gentamicin sulfate (CAMBREX 17-518Z), and 10 % heat inactivated fetal bovine serum (PAA A15-144) at 37°C in high density polyethylene flasks (NUNC 156340). For the antiproliferative assay, the test substances were dissolved in DMSO before being diluted in DMEM. The adherent cells were harvested at the logarithmic growth phase after soft trypsinization, using 0.25 % trypsin in PBS containing 0.02 % EDTA (Biochrom KG L 2163). For each experiment, approximately 10.000 cells were seeded with 0.1 mL culture medium per well of the 96-well microplates (NUNC 167008). For the cytotoxic assay, HeLa cells were pre-incubated for 48 hours without the test substances. The dilutions of the compounds were carried out carefully on the subconfluent monolayers of HeLa cells after the pre-incubation time. Cells were incubated with dilutions of the test substances for 72 hours at 37 °C in a humidified atmosphere and 5 % CO_2.

For estimating the influence of chemical compounds on cell proliferation of K-562, the number of viable cells present in multi-well plates were determined via CellTiter-Blue® assay. The indicator dye resazurin was used to measure the metabolic capacity of cells as an indicator of cell viability. Viable cells of untreated control retain the ability to reduce resazurin into resorufin, which is highly fluorescent. Nonviable cells rapidly lose metabolic capacity, do not reduce the indicator dye, and consequently do not generate a fluorescent signal. Under these experimental conditions, the signal from the CellTiter-Blue® reagent is proportional to the number of viable cells. The adherent HUVEC and HeLa cells were fixed by glutaraldehyde and stained with a 0.05 % solution of methylene blue for 15 min. After gentle washing the stain

was eluted with 0.2 mL of 0.33 N HCl in the wells. The optical densities were measured at 660 nm in SUNRISE microplate reader (TECAN). The GI_{50} and CC_{50} values were defined as being where the dose response curve intersected the 50% line, compared to untreated control. The comparisons of the different values were performed with software Magellan (TECAN).

References

Abdou R, Scherlach K, Dahse HM, Sattler I, Hertweck C. 2010. Botryorhodines A–D, antifungal and cytotoxic depsidones from *Botryosphaeria rhodina*, an endophyte of the medicinal plant *Bidens pilosa*. Phytochem. 71: 110–116.

Bonev B, Hooper J, Parisot J. 2008. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J Antimicrob Chemother. 61: 1295–1301.

Wayne PA. 2002. Reference method for broth dilution susceptibility testing of filamentous fungi; Approved Standard. M38-A, USA, NCCls, p. 22.

Table S1. Summary of ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for 14-hydroxyterezine D **1** and terezine E **2** in acetone-d_6 at 298 K.

Position	14-hydroxyterezine D **1**		Terezine E **2**	
	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)
1		8.11, s		8.11, s
2	125.0, CH	7.11, s	124.4, CH	7.13, s
3	108.8, C		110.7, C	
3a	127.7, C		128.4, C	
4	119.7, CH	6.79, d (7.8)	119.7, CH	6.86, d (8.1)
5	118.7, CH	6.86, t (7.9)	118.7, CH	6.91, dd (7.9, 8.2)
6	115.9, CH	7.47, d (7.8)	115.9, CH	7.61, d (7.8)
7	124.8, C		124.8, C	
7a	134.5, C		133.1, C	
8	29.0, CH_2	3.50, d (7.1)	30.1, CH_2	3.52, d (7.0)
9	121.1, CH	5.38, t (7.3)	123.1, CH	5.48, t (7.1)
10	135.7, C		135.8, C	
11	25.8, CH_3	1.71, s	25.8, CH_3	1.71, s
12	17.8, CH_3	1.72, s	18.24, CH_3	1.73, s
1′	25.7, CH_2	3.45, dd (14.3, 3.5)	19.4, CH_2	4.01, dd (14.5, 3.7)
2′	64.2, CH	4.49, t (3.6)	153.4, C	
3′	165.9, C		163.6, C	
5′	48.7, CH	3.78, q	48.5, CH	4.48, q
6′	162.4, C		174.2, C	
8′	19.1, CH_3	0.21, d (6.8)	18.22, CH_3	1.34, d (6.8)

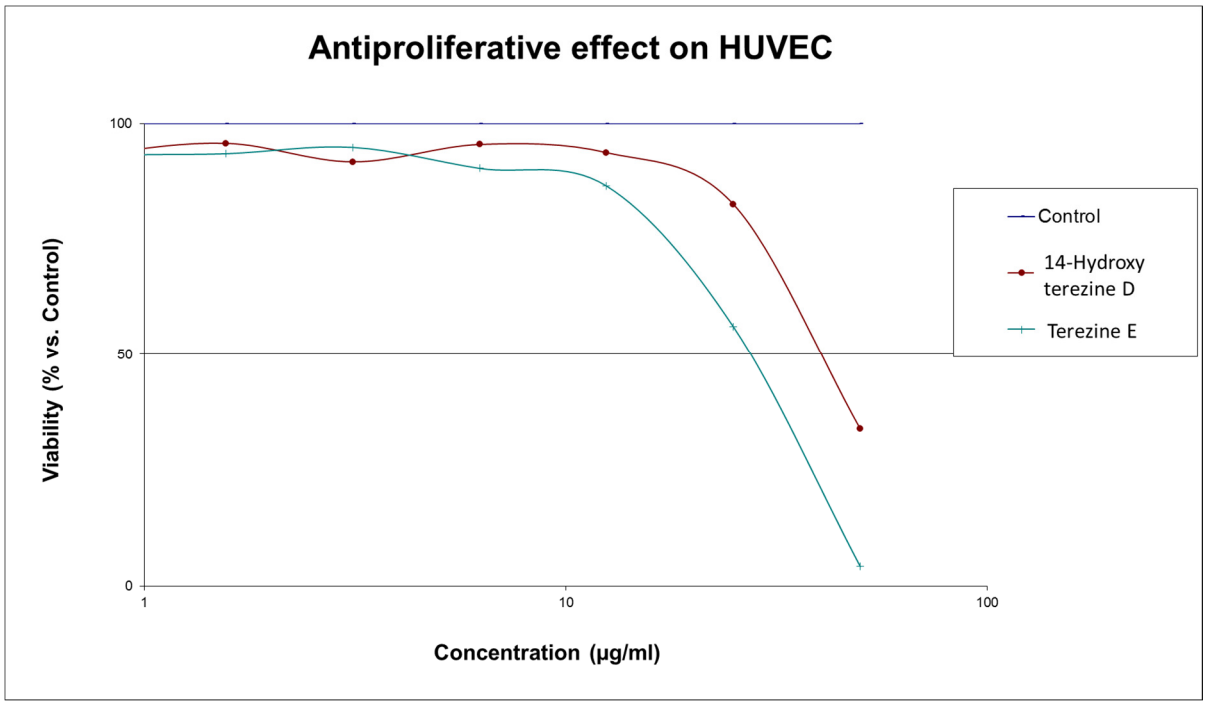

Figure S1. The antiproliferative effect of the isolated compounds on HUVEC cell line.

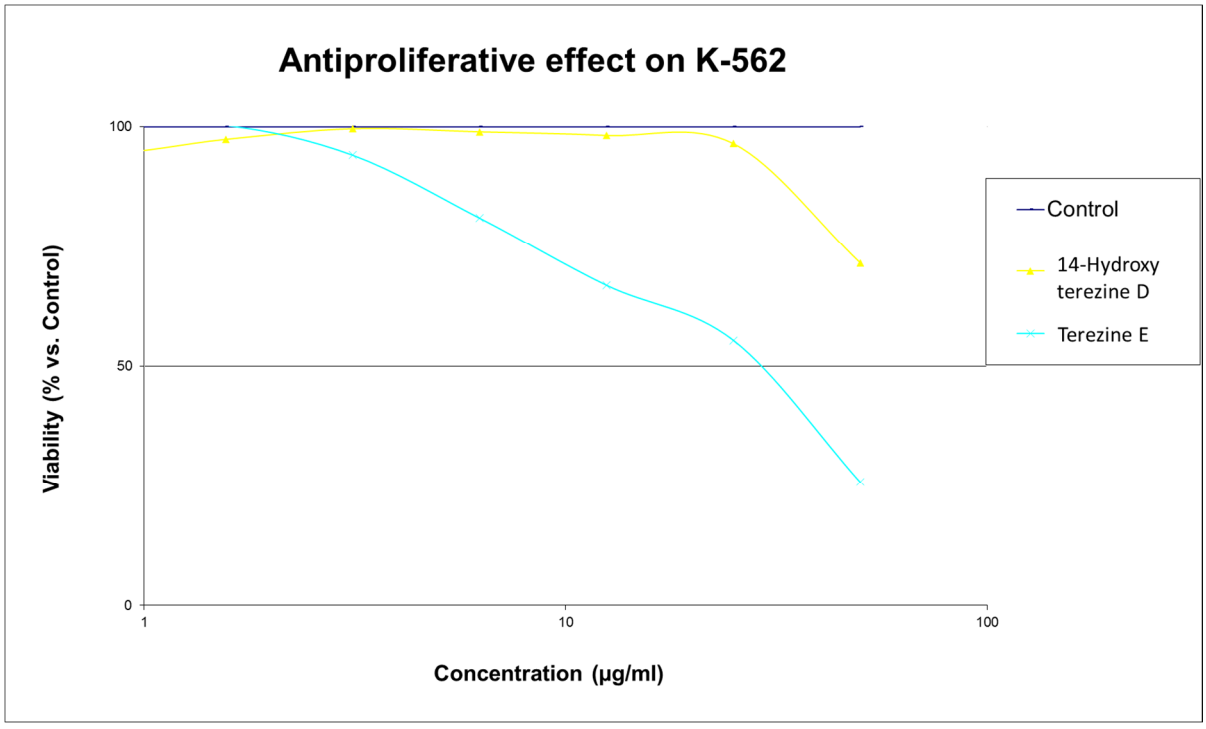

Figure S2. The antiproliferative effect of the isolated compounds on K-562 cell line.

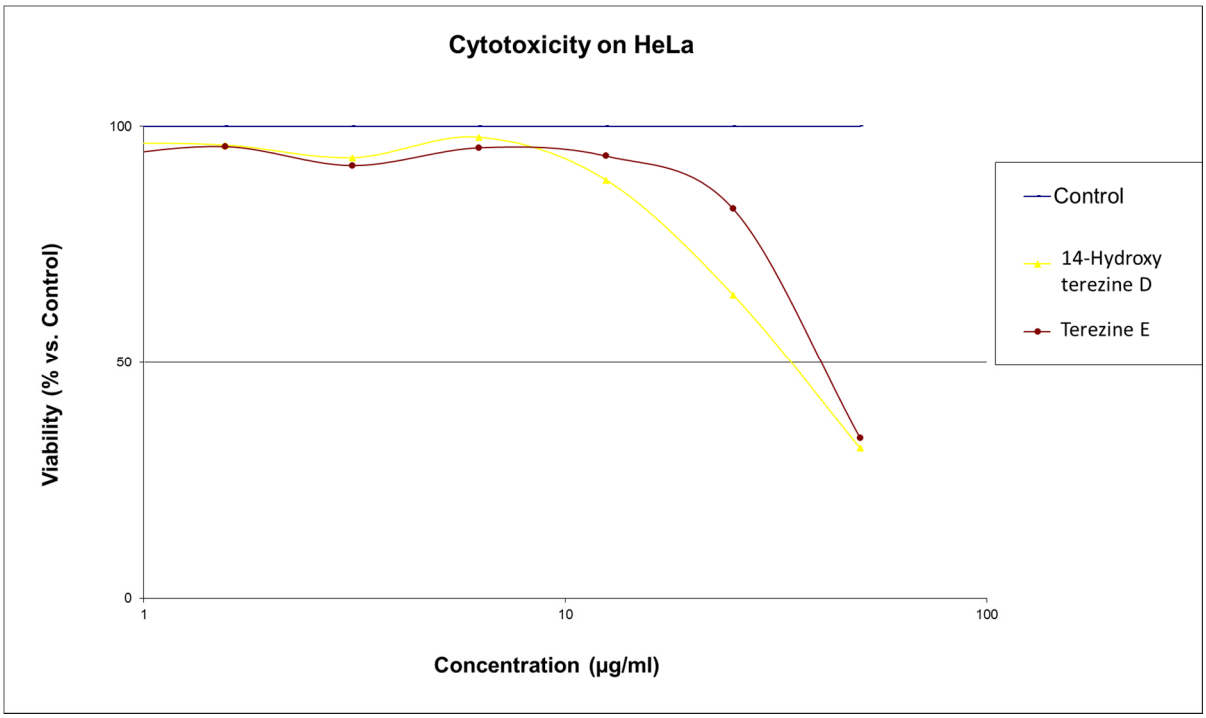

Figure S3. The cytotoxic effect of the isolated compounds on HeLa cell line.

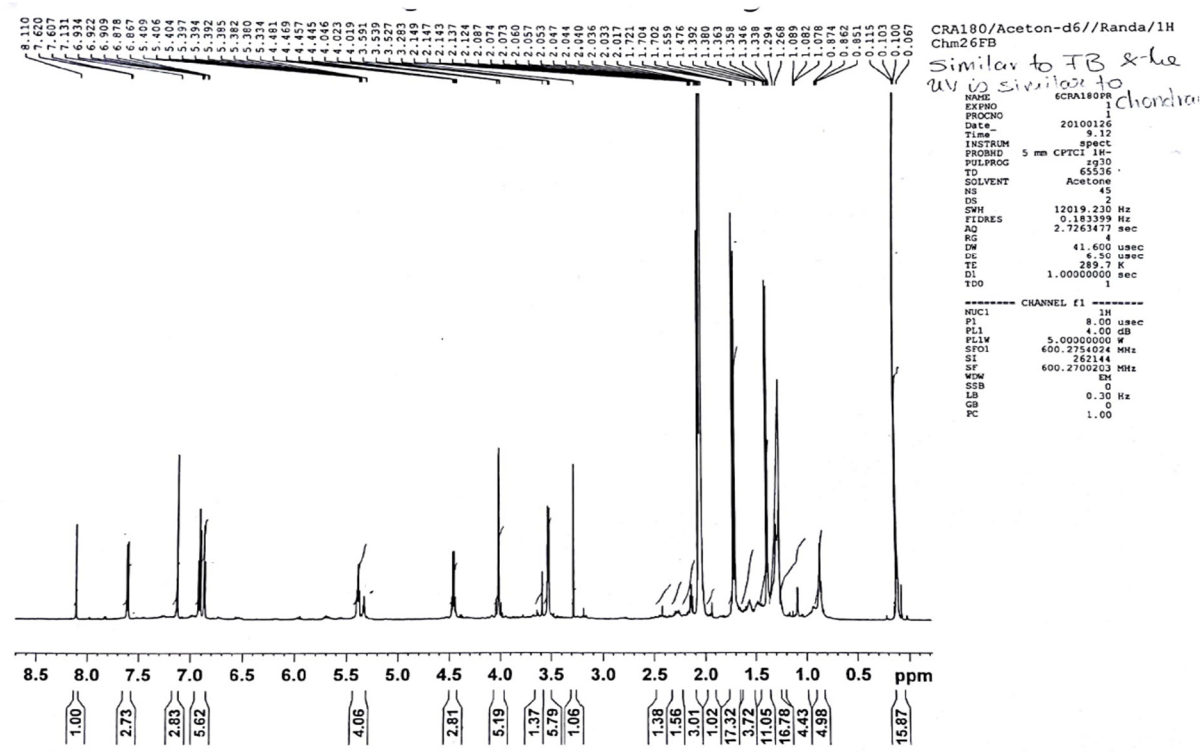

Figure S4. ^{1}H NMR spectrum of Terezine E **2** in acetone-d_6.

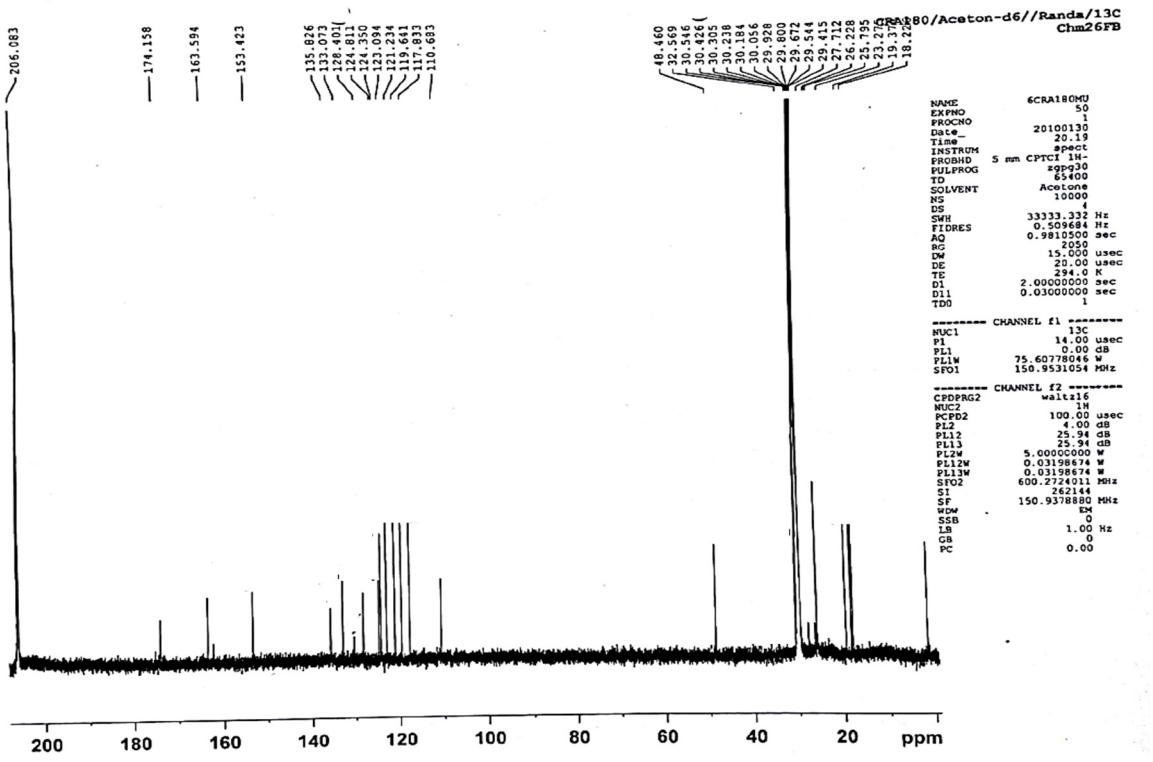

Figure S5. ^{13}C NMR spectrum of Terezine E **2** in acetone-d_6.

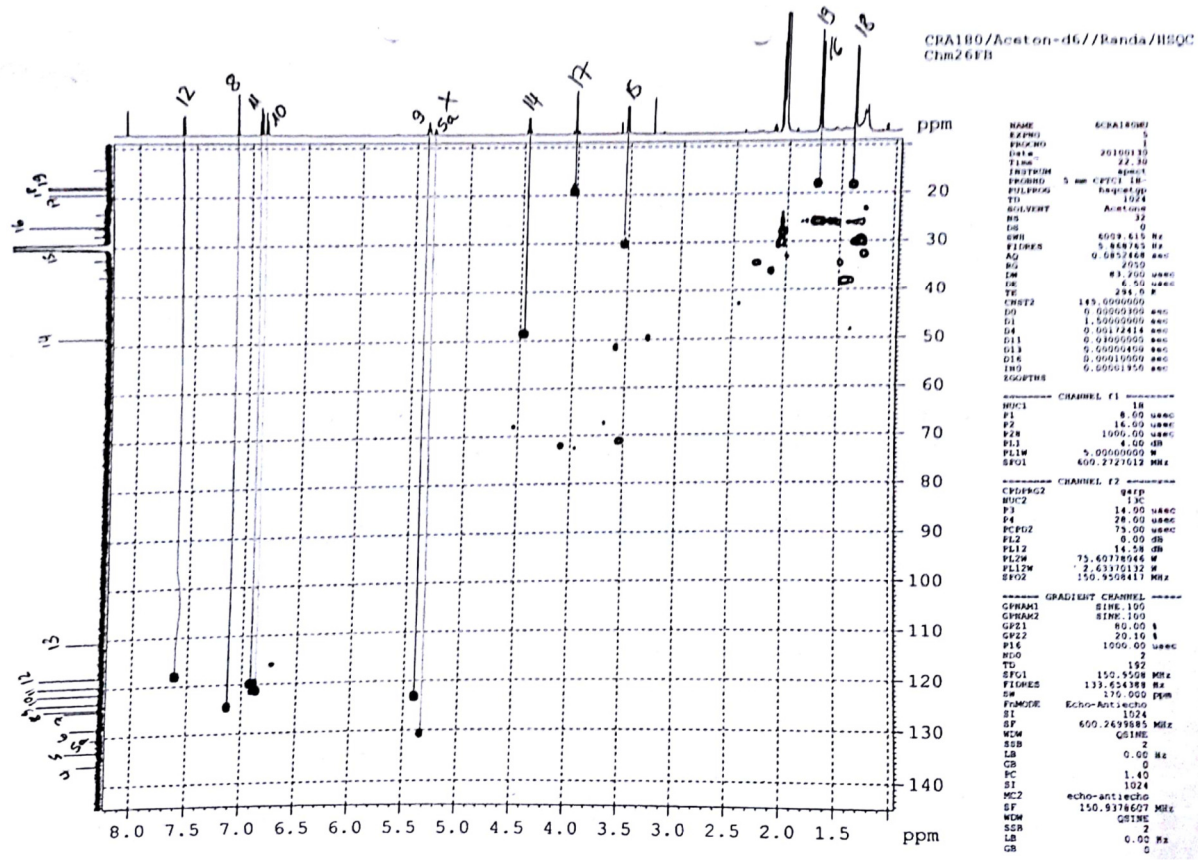

Figure S6. HSQC spectrum of Terezine E **2** in acetone-d_6.

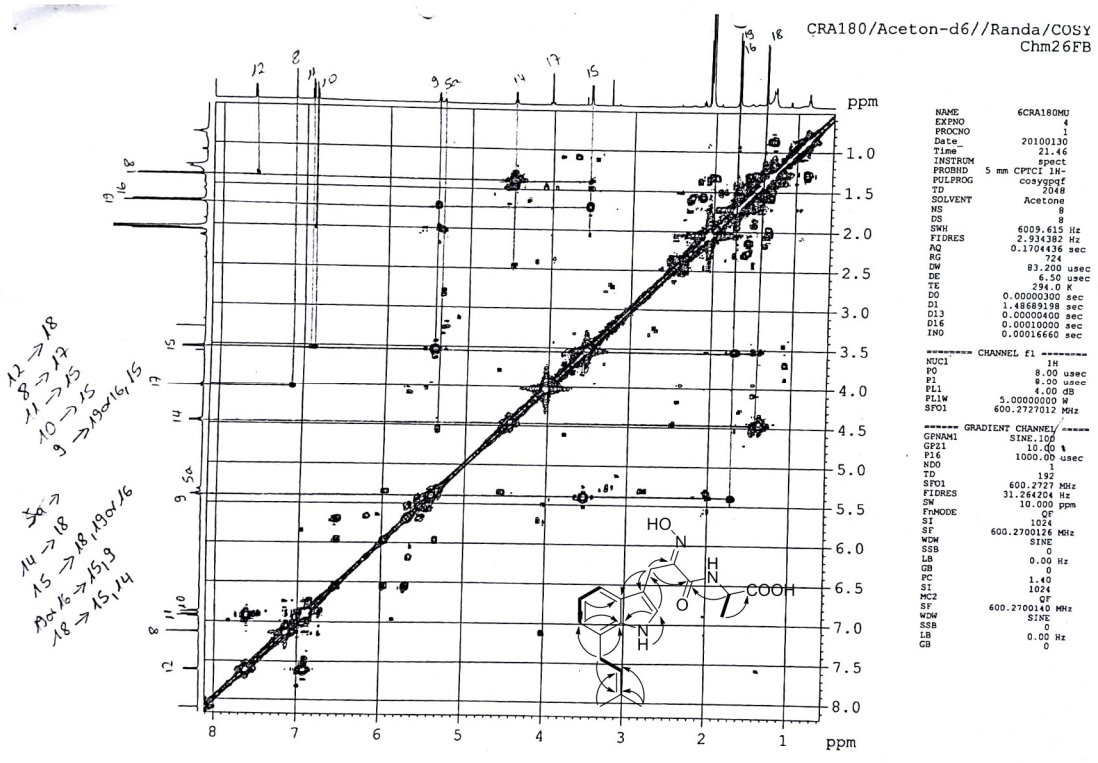

Figure S7. COSY spectrum of Terezine E **2** in acetone-d_6.

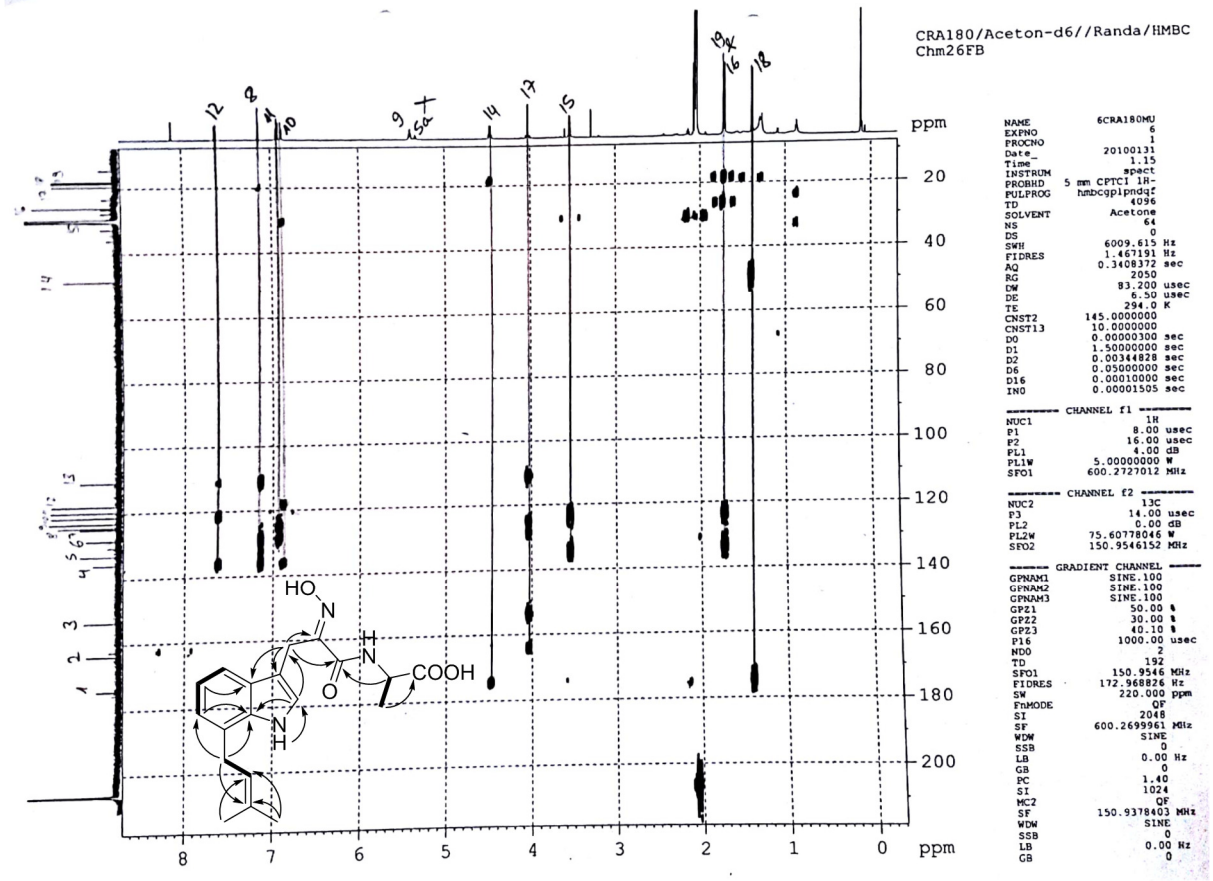

Figure S8. HMBC spectrum of Terezine E **2** in acetone-d_6.

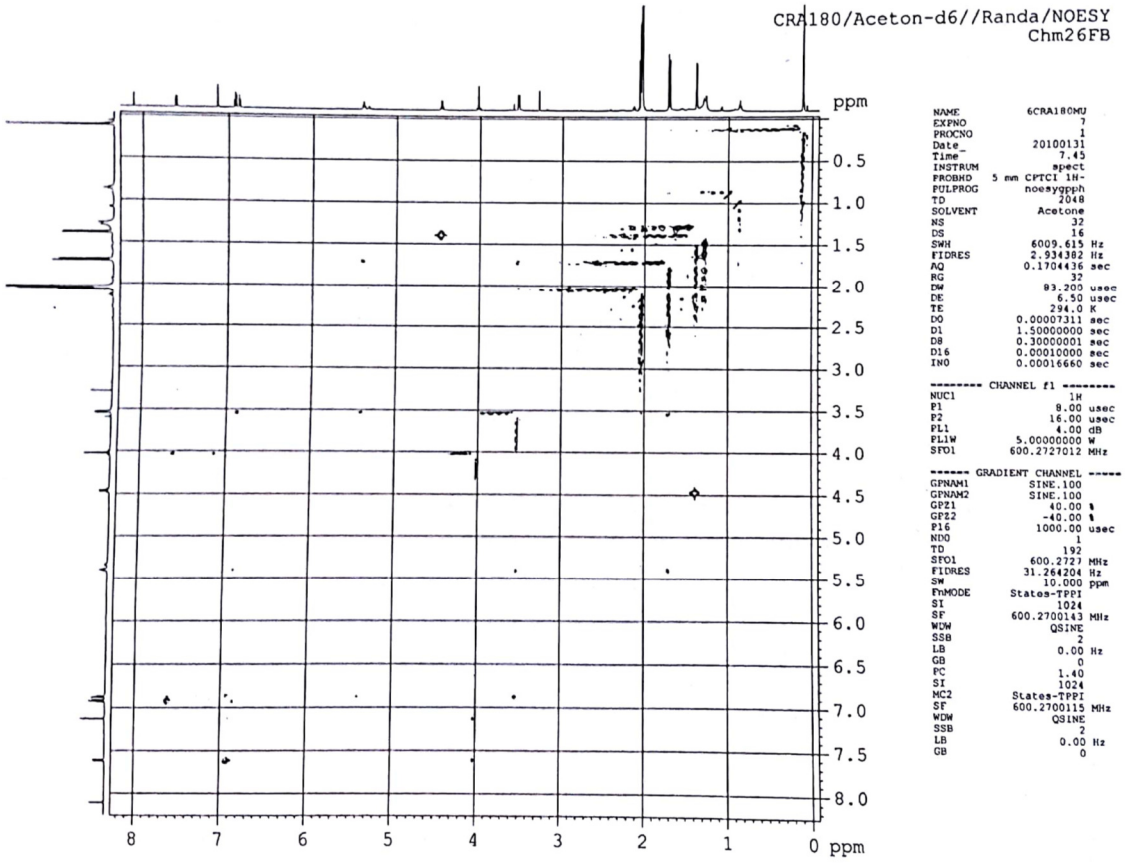

Figure S9. NOESY spectrum of Terezine E **2** in acetone-d_6.