Supporting Information

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Two New Diketopiperazine Alkaloids from a Deep-soil Derived Fungus *Penicillium simplicissimum* GZWMJZ-1612

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Table of Contents **Page**

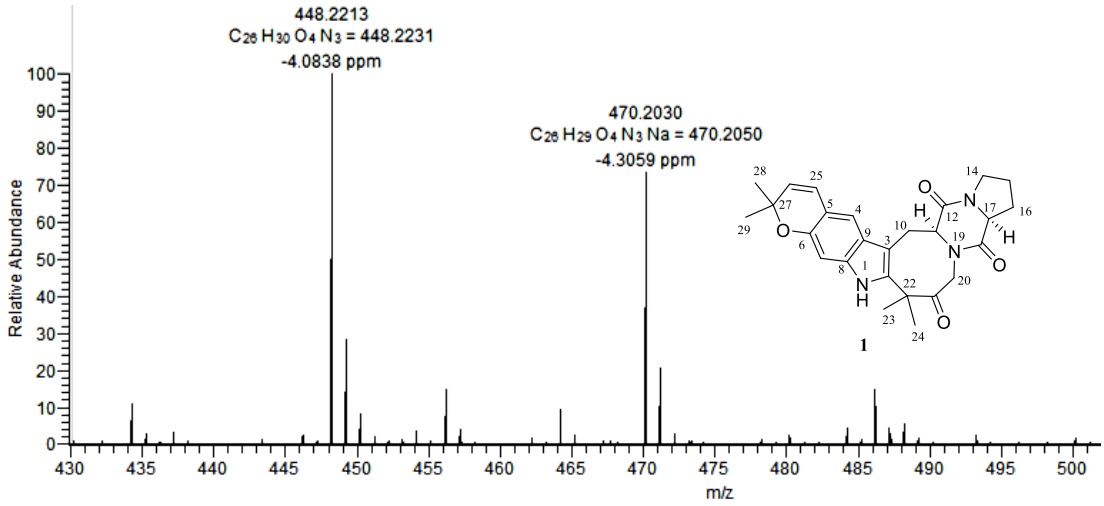


Figure S1: HRESIMS spectrum of **1** (simplicamides A)

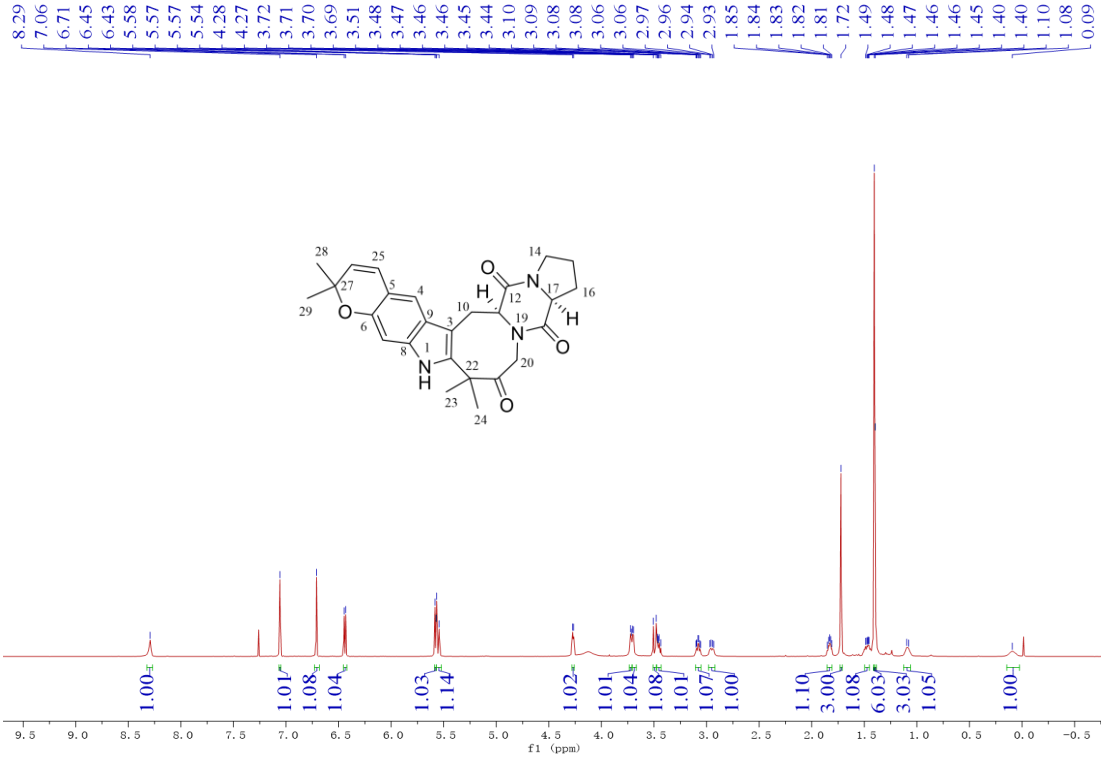


Figure S2: ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **1**

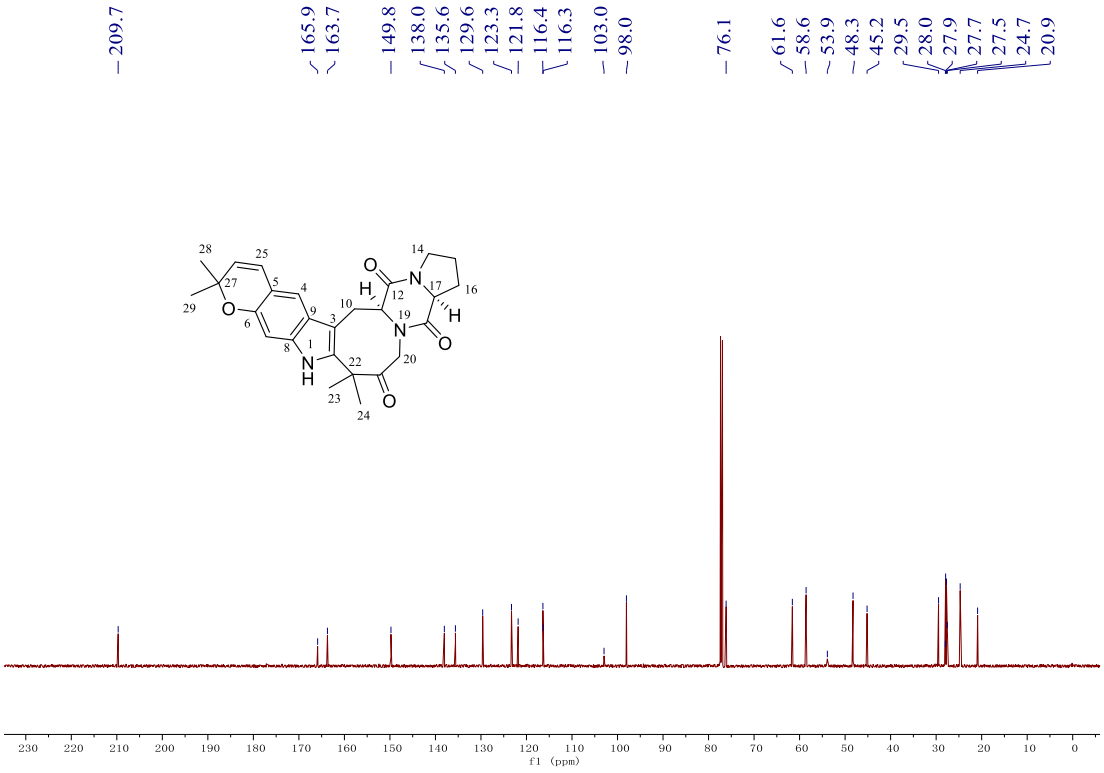


Figure S3: ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of **1**

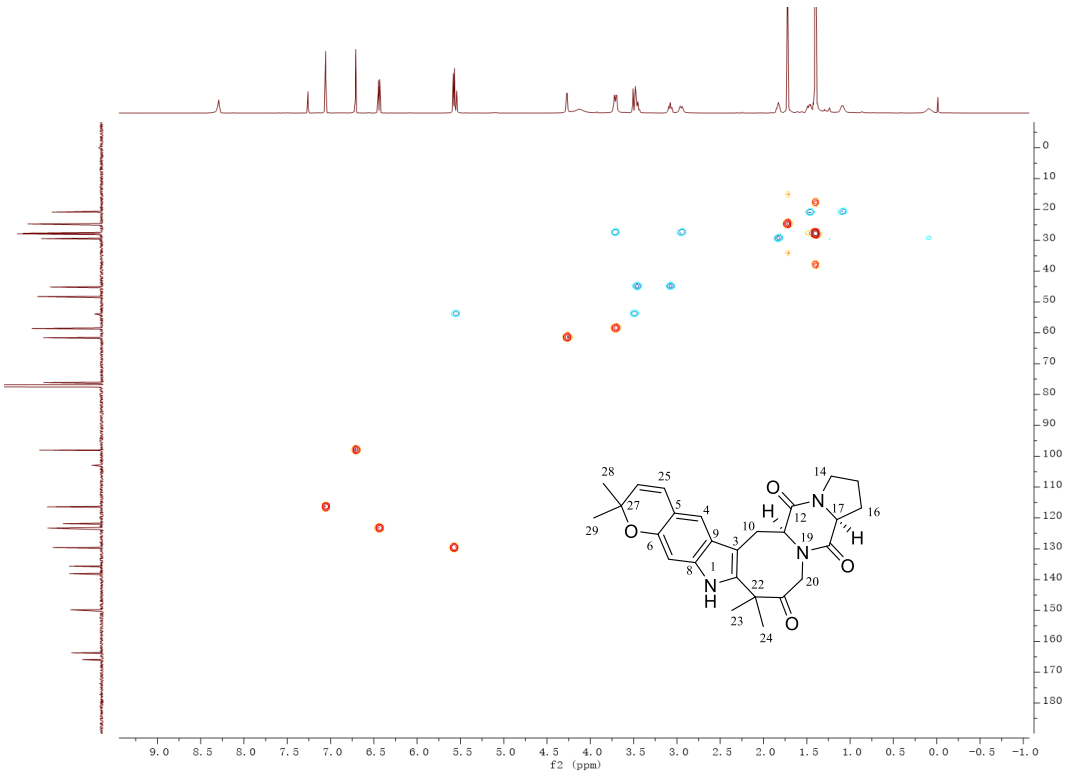


Figure S4: HSQC spectrum of **1** in $CDCl_3$

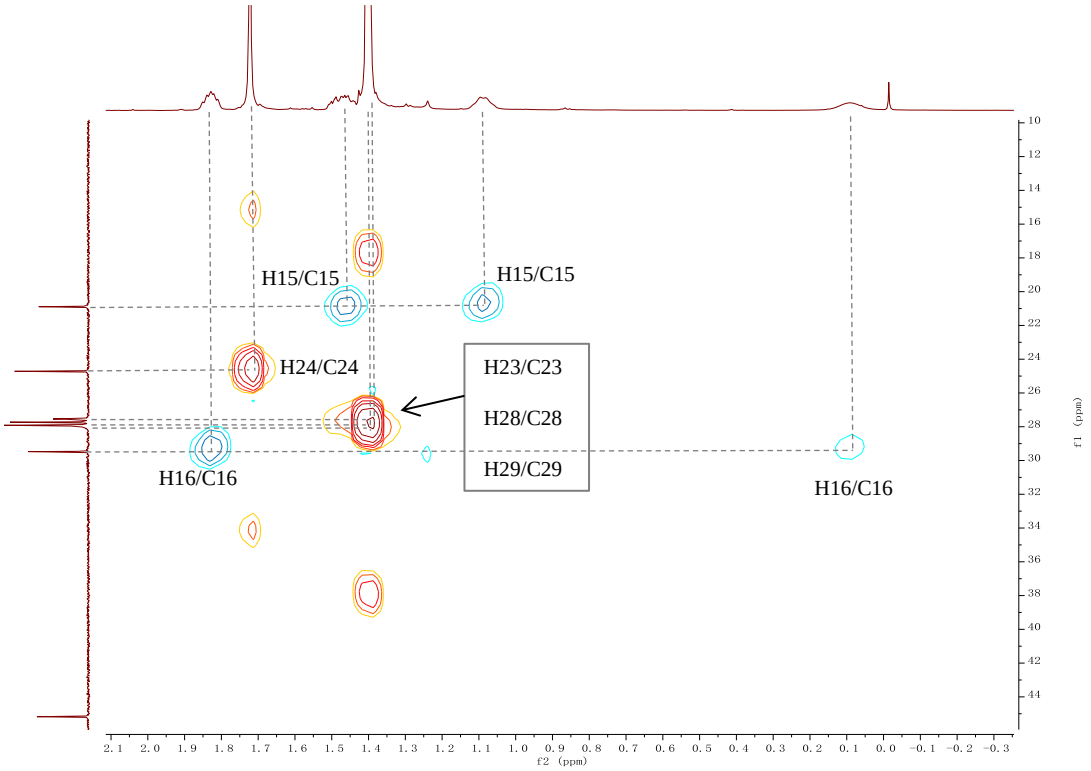


Figure S5: HSQC spectrum of **1** in $CDCl_3$ (From δ_C 10 ppm to δ_C 45 ppm)

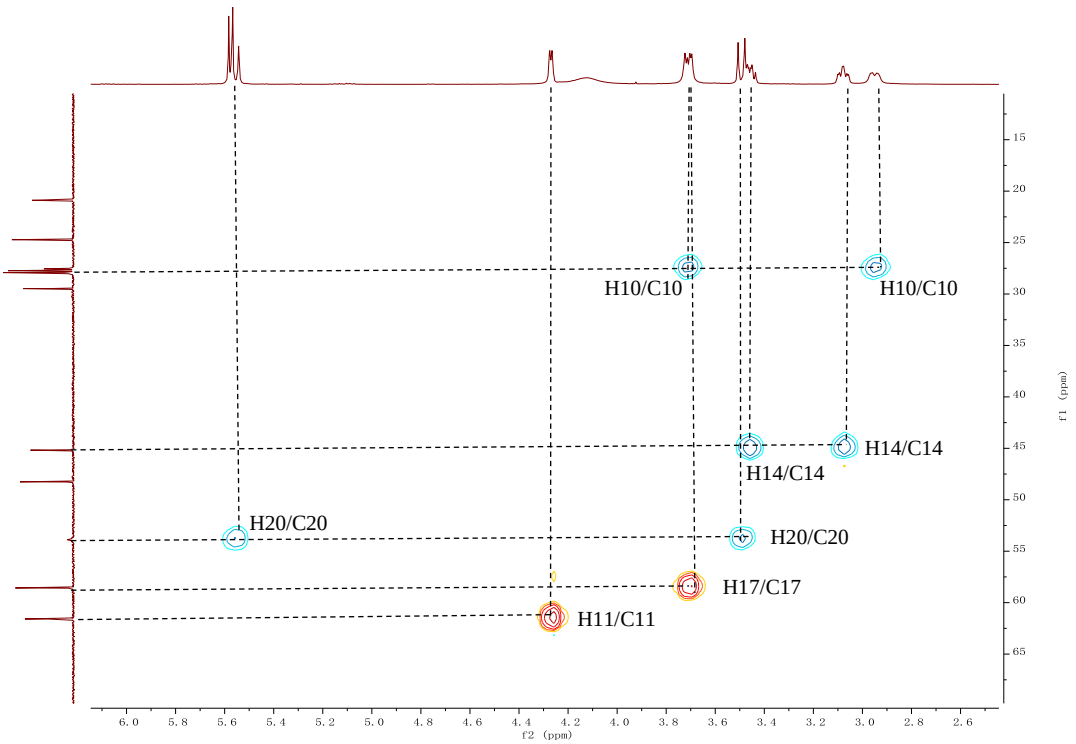


Figure S6: HSQC spectrum of **1** in $CDCl_3$ (From δ_C 15 ppm to δ_C 65 ppm)

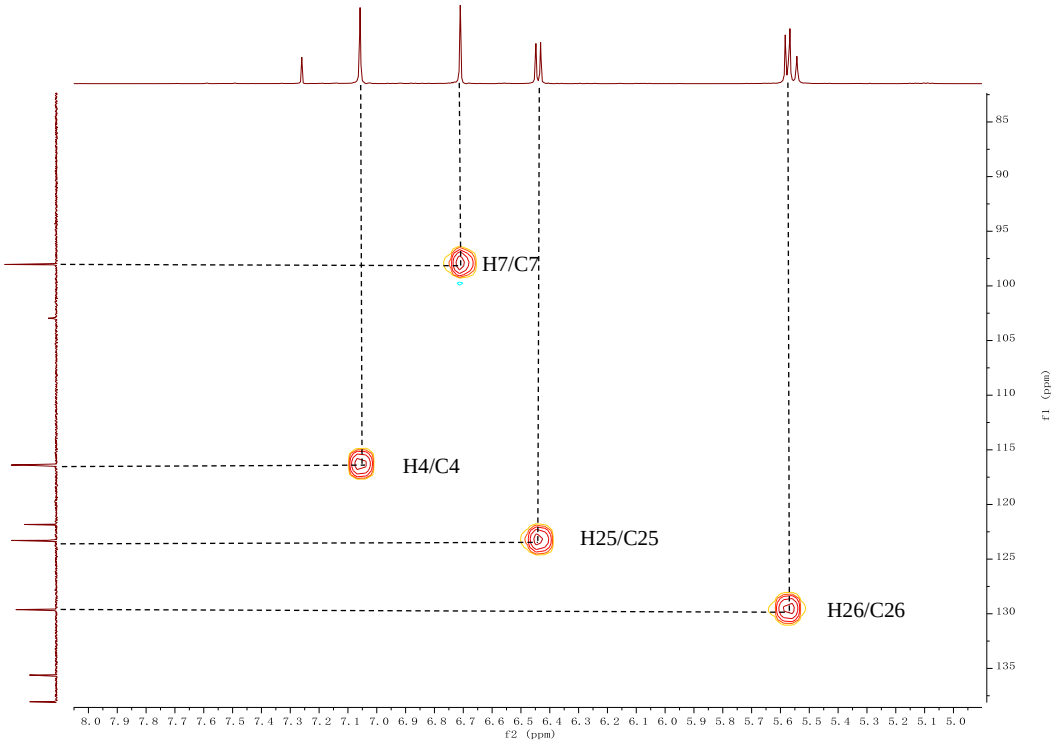


Figure S7: HSQC spectrum of **1** in $CDCl_3$ (From δ_C 85 ppm to δ_C 135 ppm)

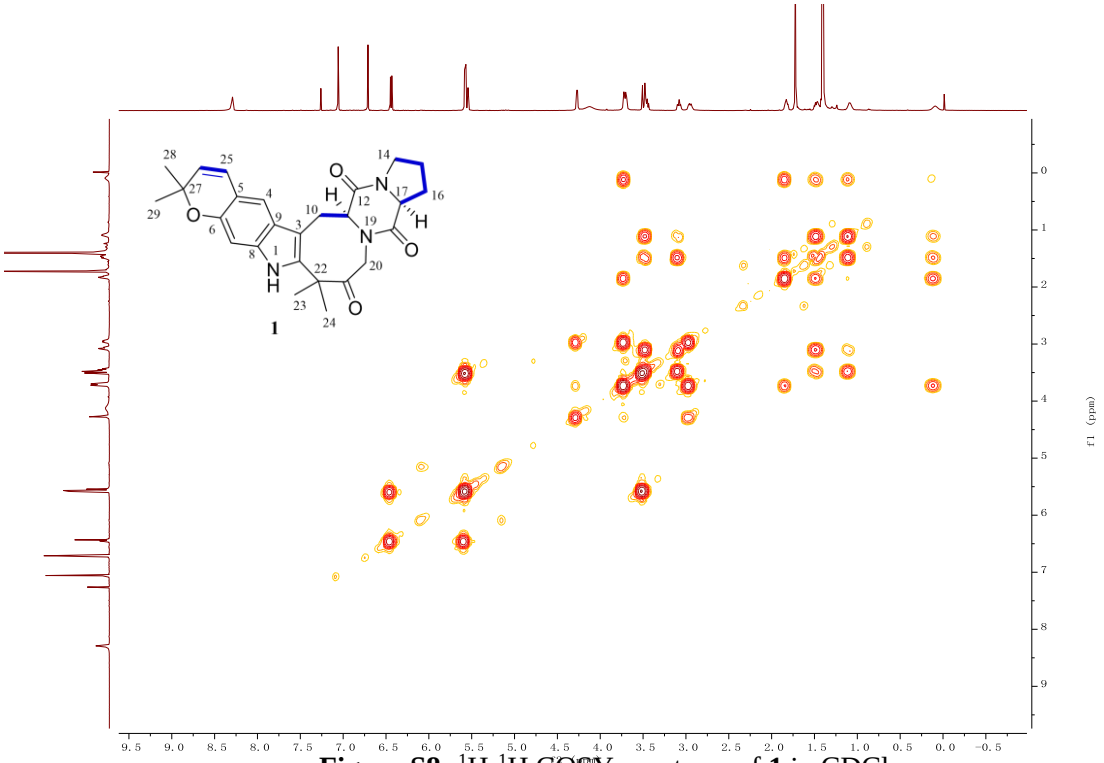


Figure S8: ^1H-^1H COSY spectrum of **1** in $CDCl_3$

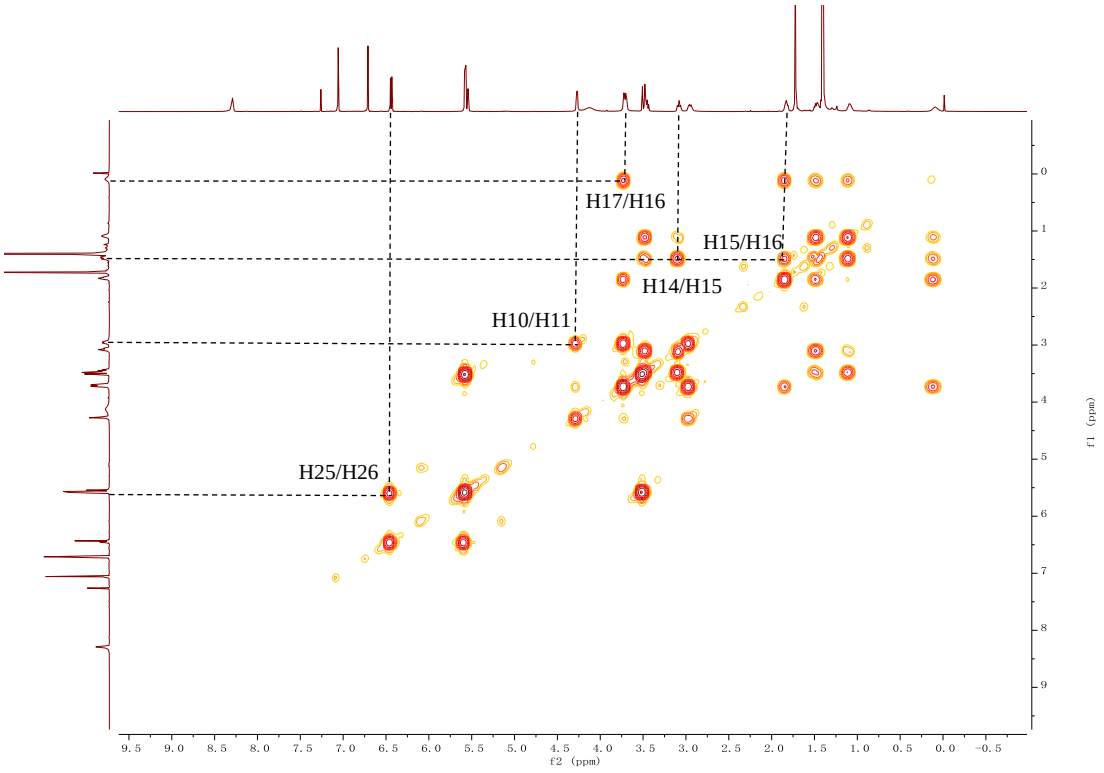


Figure S9: ^{1}H-^{1}H COSY spectrum of **1** in $CDCl_3$ (From δ_H 0.1 ppm to δ_H 9.0 ppm)

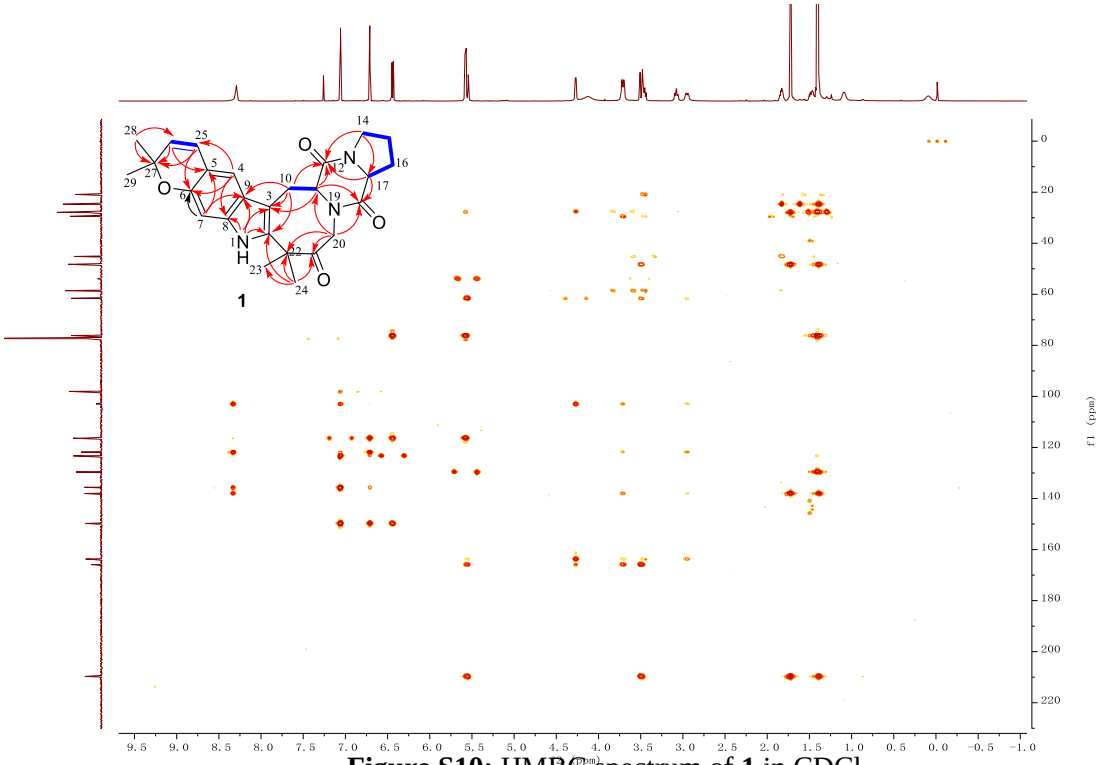


Figure S10: HMBC spectrum of **1** in $CDCl_3$

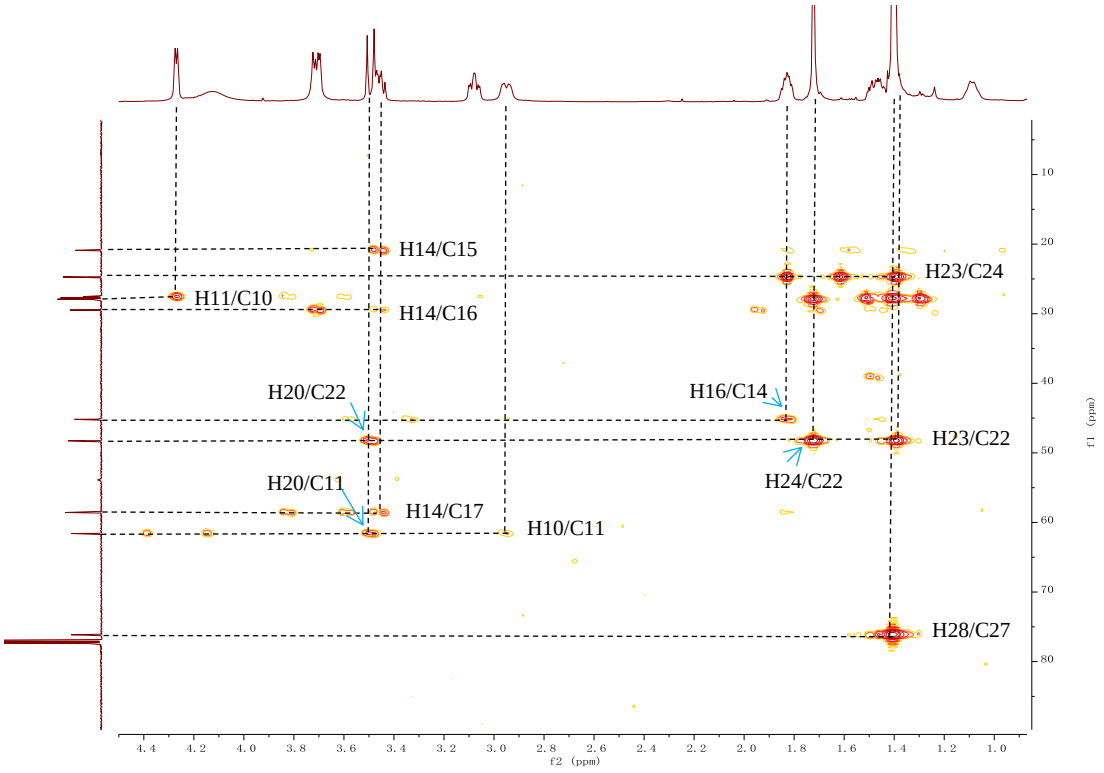


Figure S11: HMBC spectrum of **1** in $CDCl_3$ (From δ_C 10 ppm to δ_C 80 ppm)

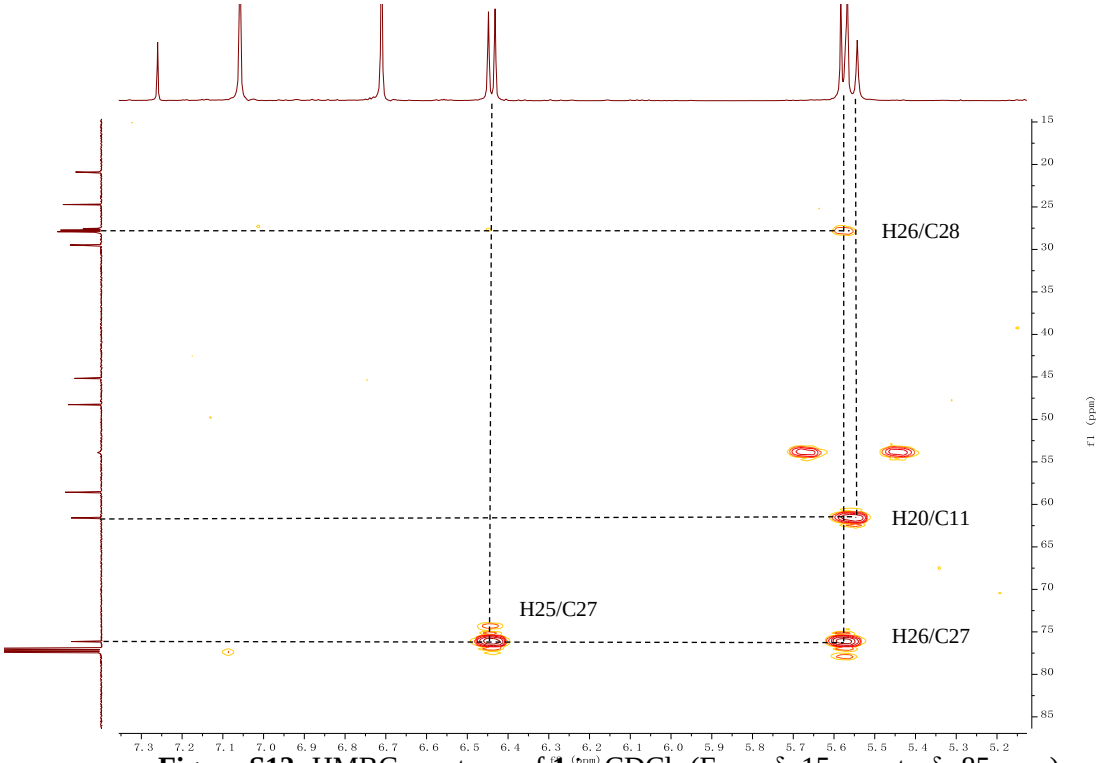


Figure S12: HMBC spectrum of **1** in $CDCl_3$ (From δ_C 15 ppm to δ_C 85 ppm)

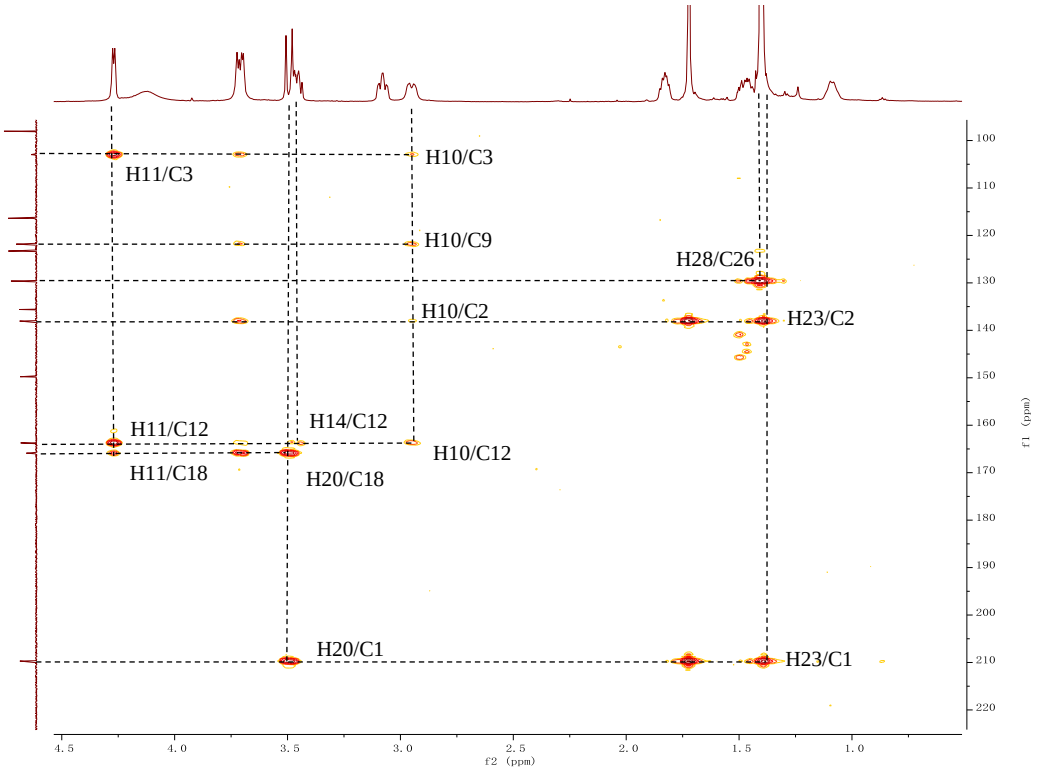


Figure S13: HMBC spectrum of **1** in $CDCl_3$ (From δ_C 100 ppm to δ_C 220 ppm)

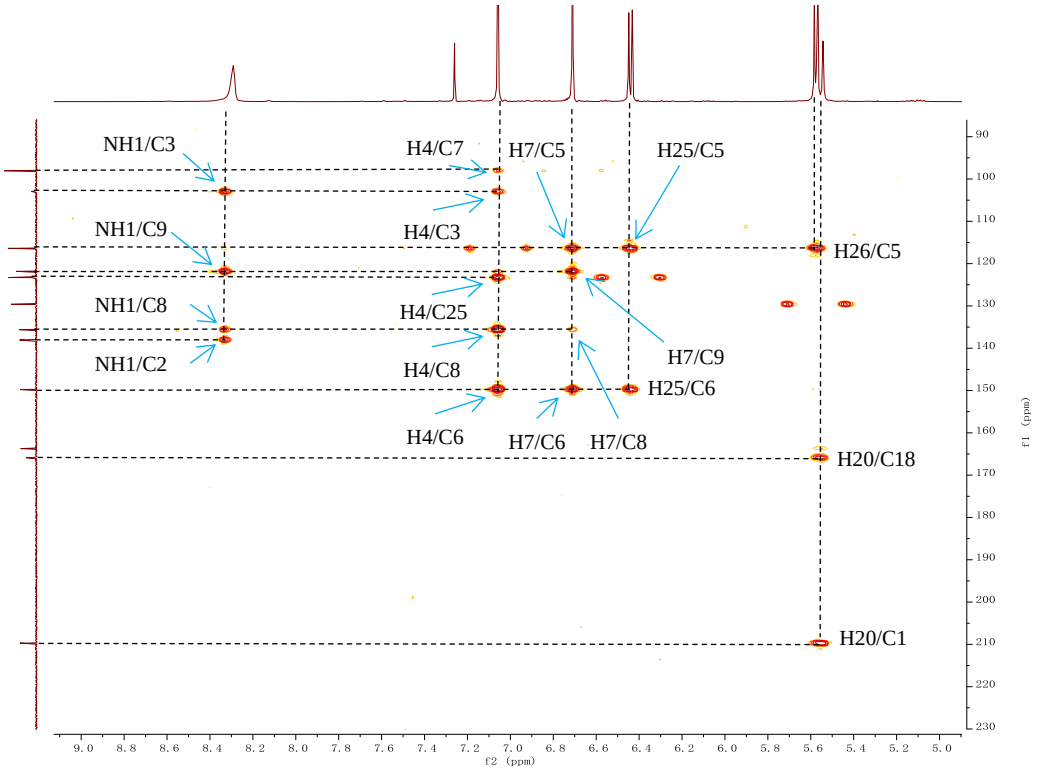


Figure S14: HMBC spectrum of **1** in $CDCl_3$ (From δ_C 90 ppm to δ_C 230 ppm)

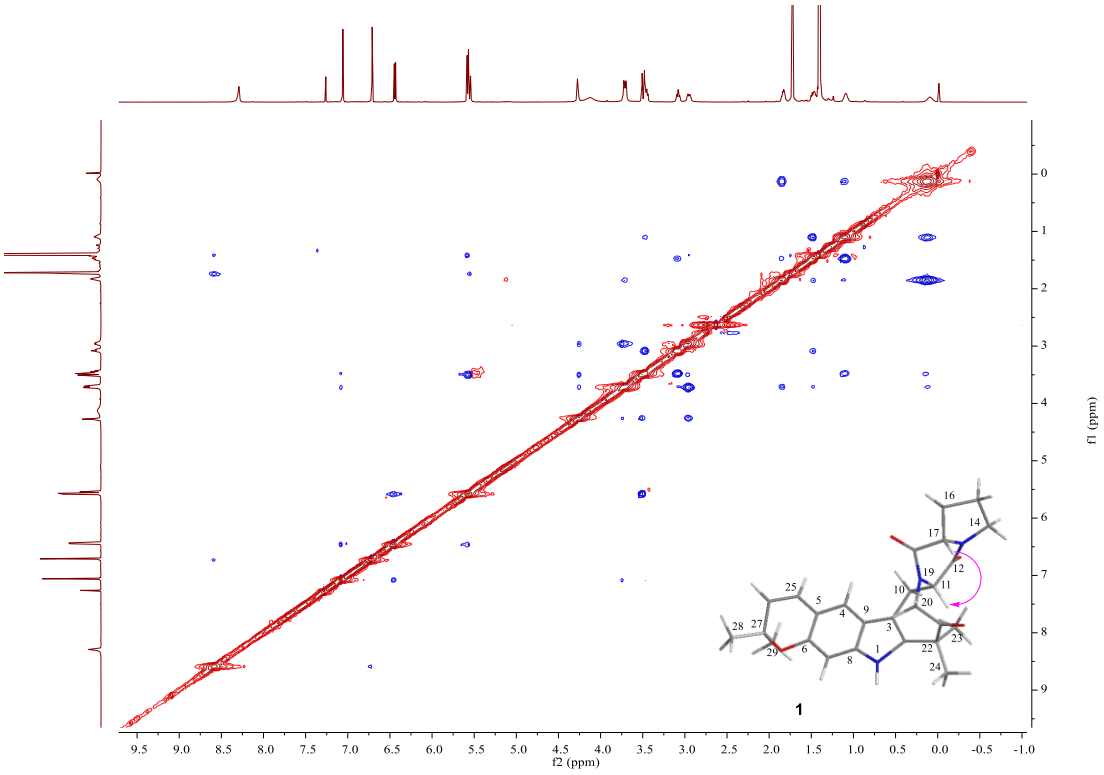


Figure S15: NOESY spectrum of **1** in $CDCl_3$

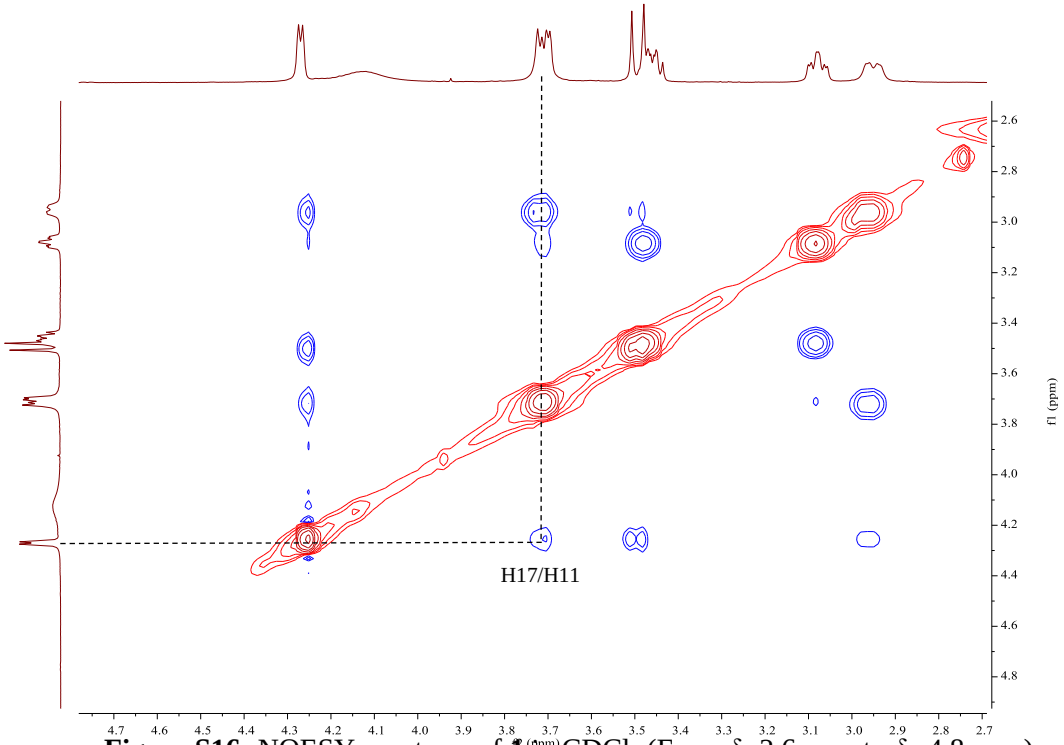


Figure S16: NOESY spectrum of **1** in $CDCl_3$ (From δ_H 2.6 ppm to δ_H 4.8 ppm)

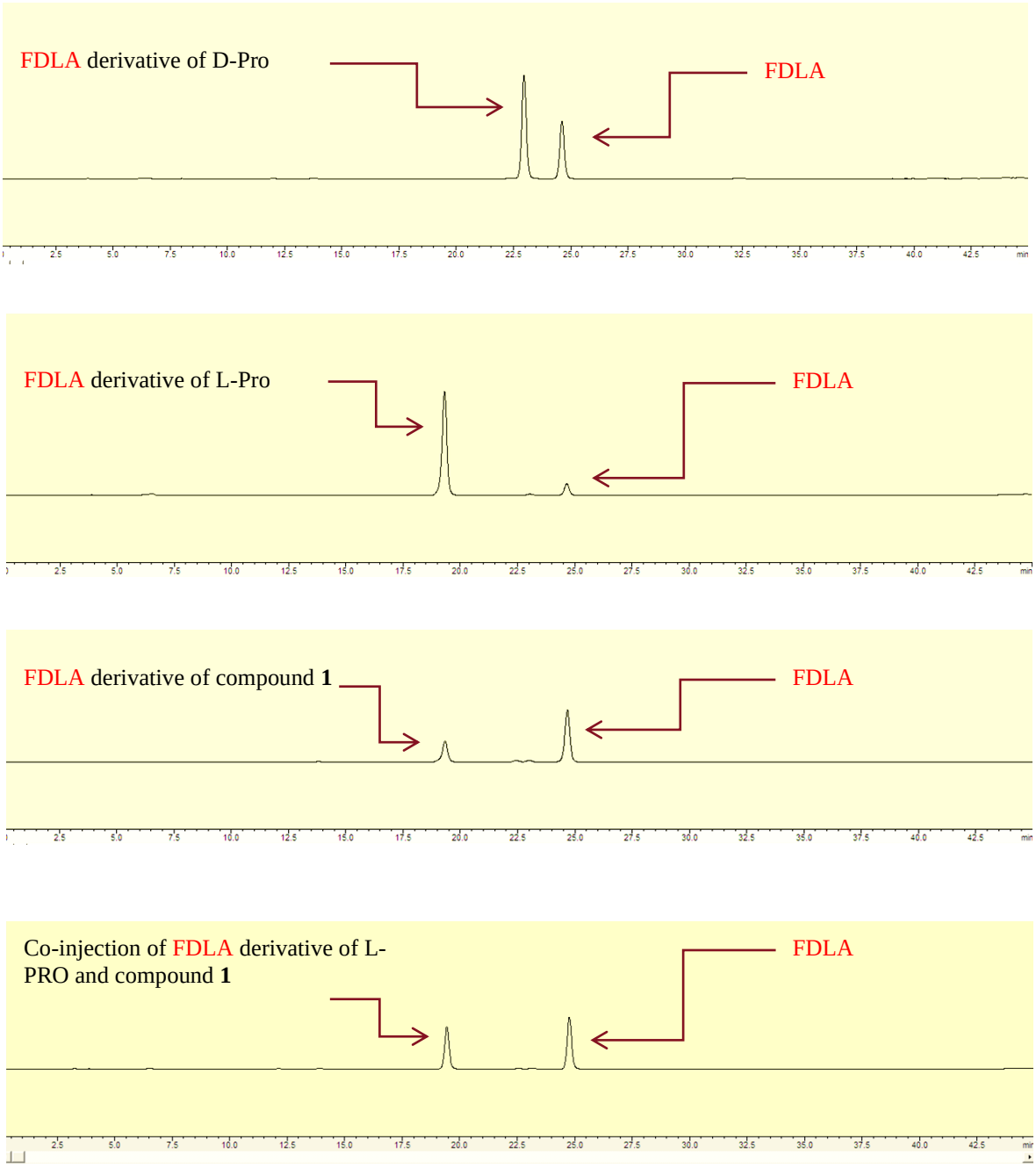


Figure S17: HPLC Analysis of D/L-FDLA Derivatives of **1**

(The t_R for the FDLA and FDLA derivatives of the standard D-Pro, L-Pro and **1** were 24.8, 22.9, 19.4 and 19.4 min, respectively.)

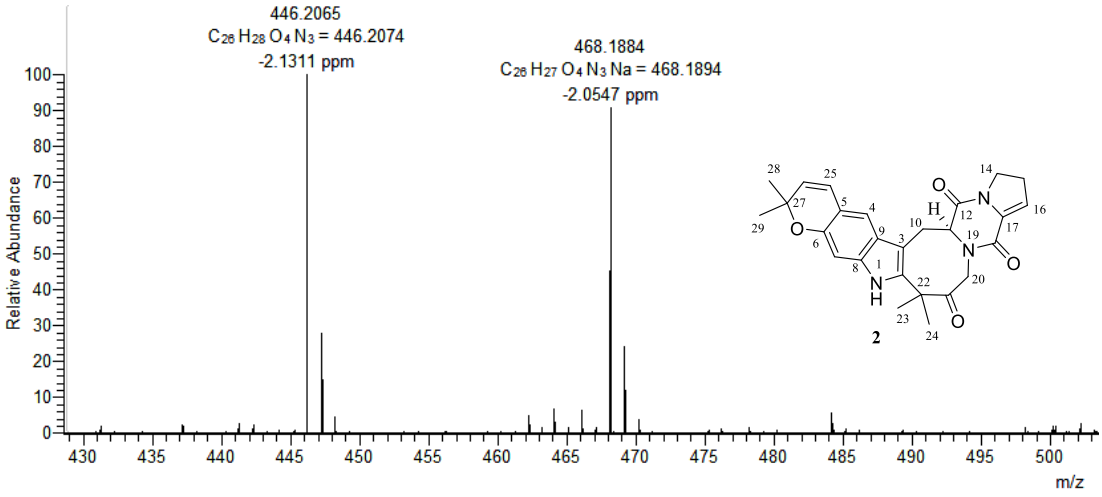


Figure S18: HRESIMS spectrum of **2** (simplicamides B)

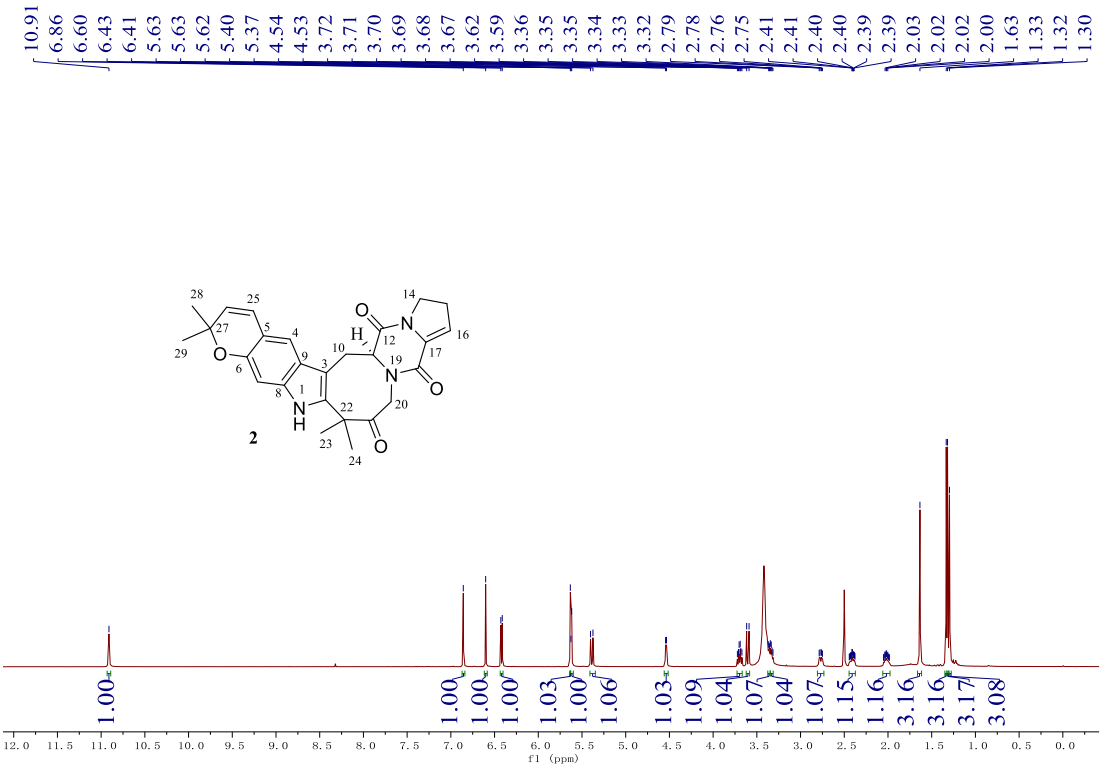


Figure S19: ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of **2**

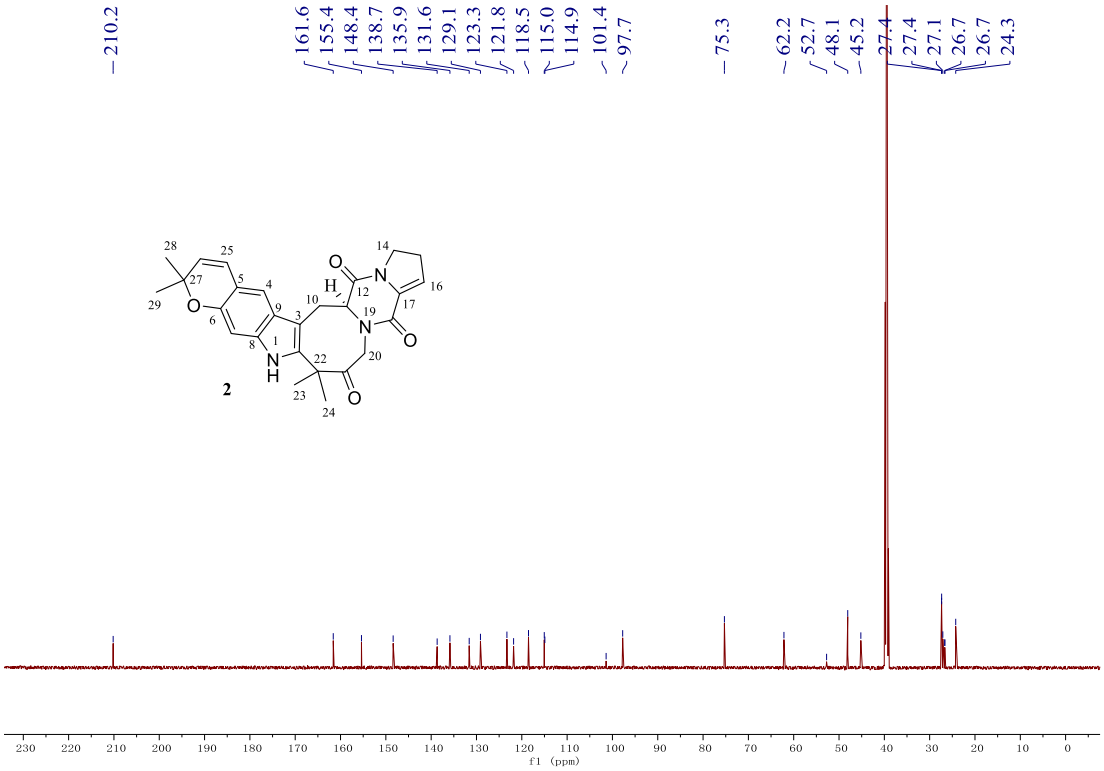


Figure S20: ^{13}C NMR (151 MHz, DMSO-d_6) spectrum of **2**

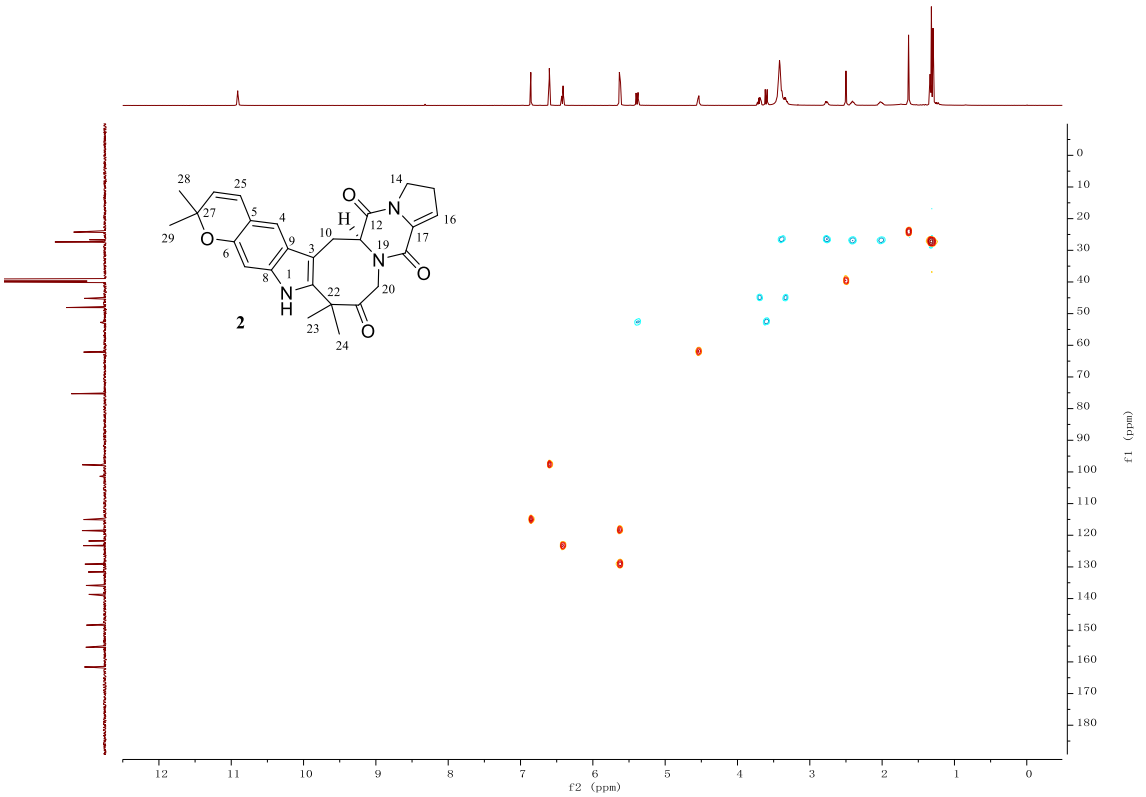


Figure S21: HSQC spectrum of **2** in DMSO-d_6

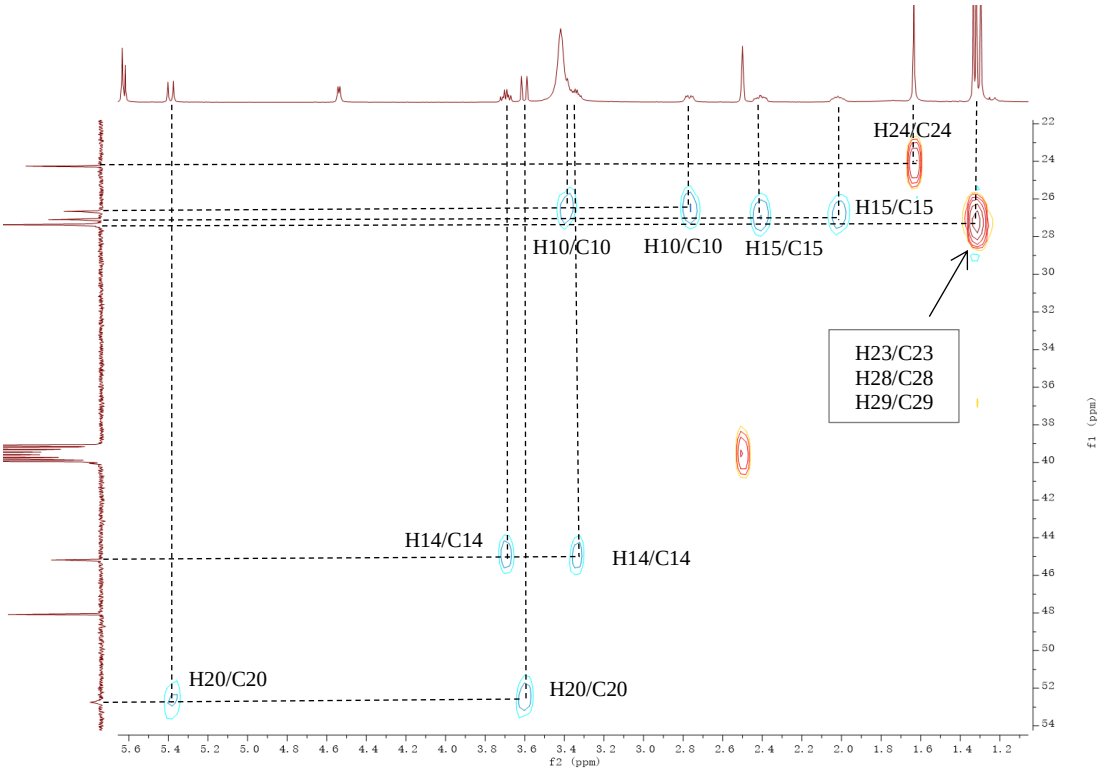


Figure S22: HSQC spectrum of **2** in DMSO-d_6 (From δ_C 22 ppm to δ_C 64 ppm)

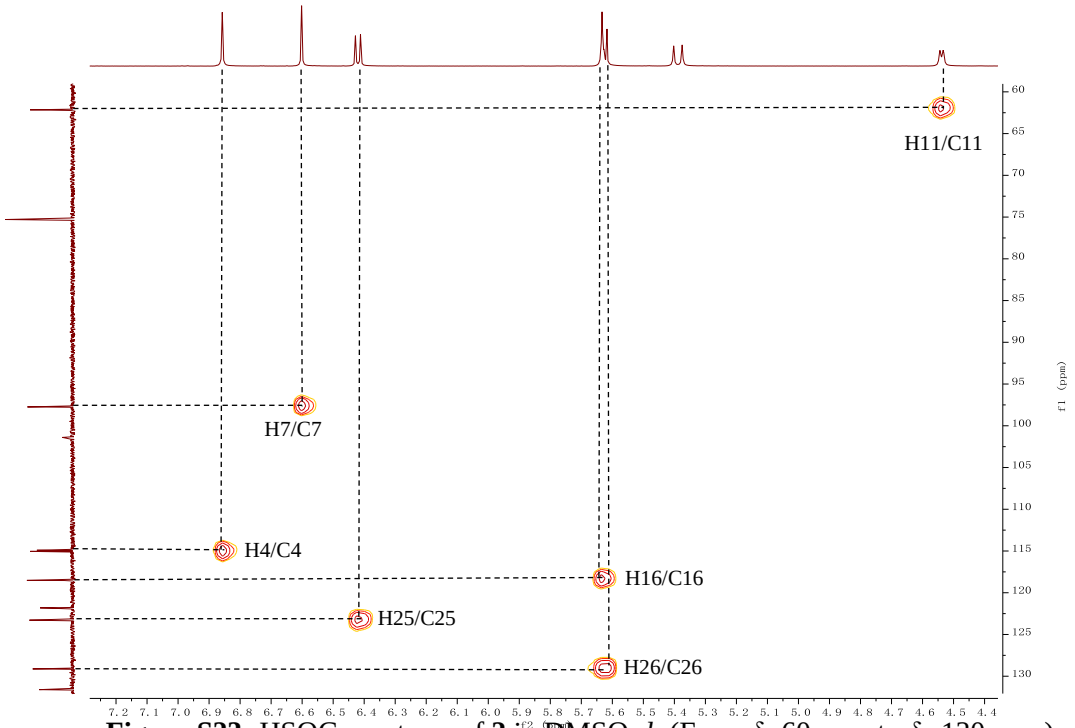


Figure S23: HSQC spectrum of **2** in DMSO-d_6 (From δ_C 60 ppm to δ_C 130 ppm)

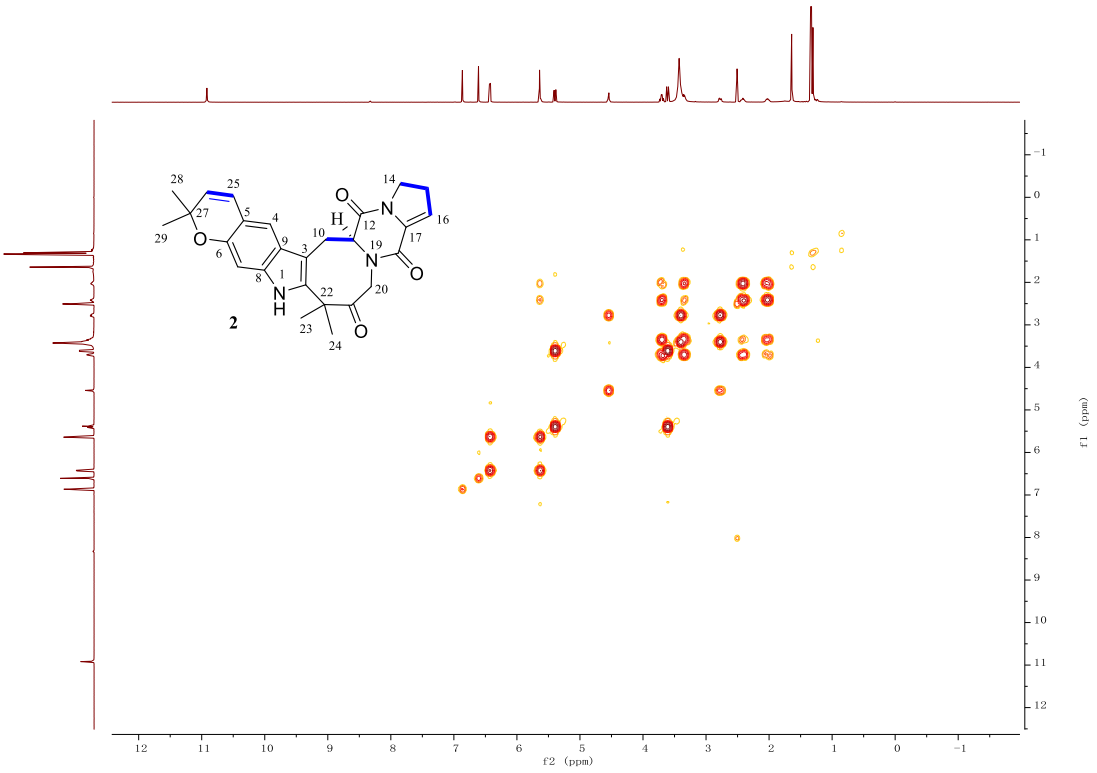


Figure S24: ^{1}H-^{1}H COSY spectrum of **2** in DMSO-d_6

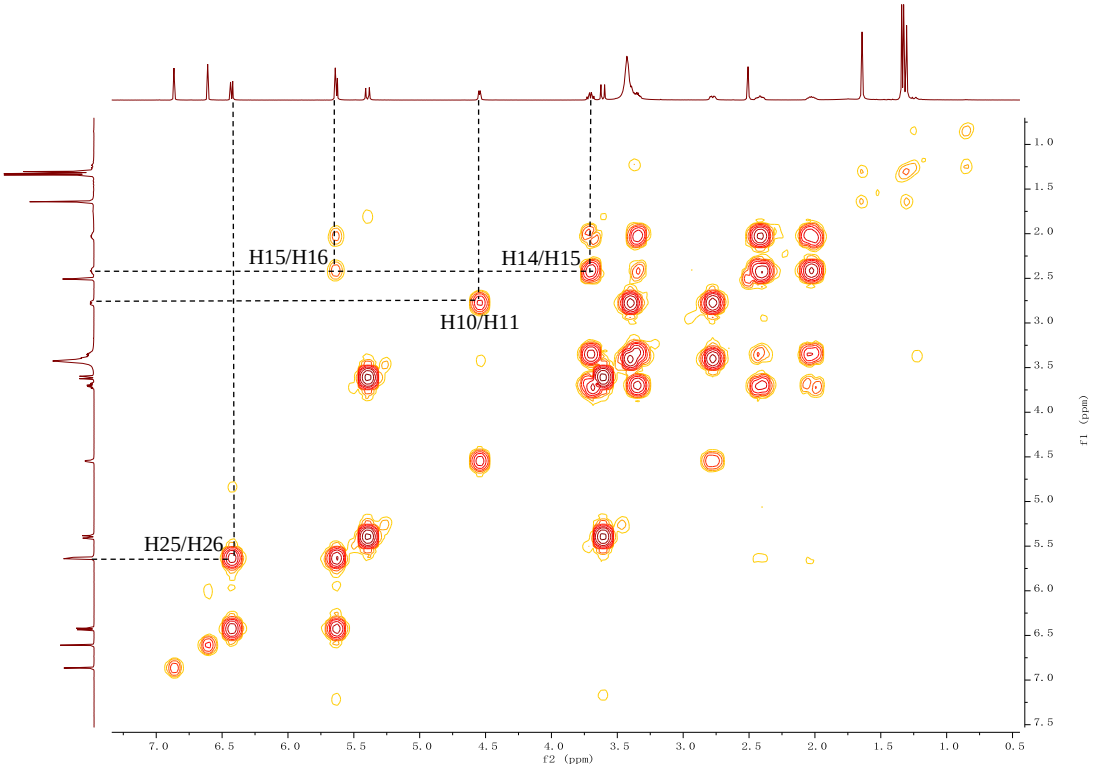


Figure S25: ^{1}H-^{1}H COSY spectrum of **2** in DMSO-d_6 (From δ_H 1.0 ppm to δ_H7.5 ppm)

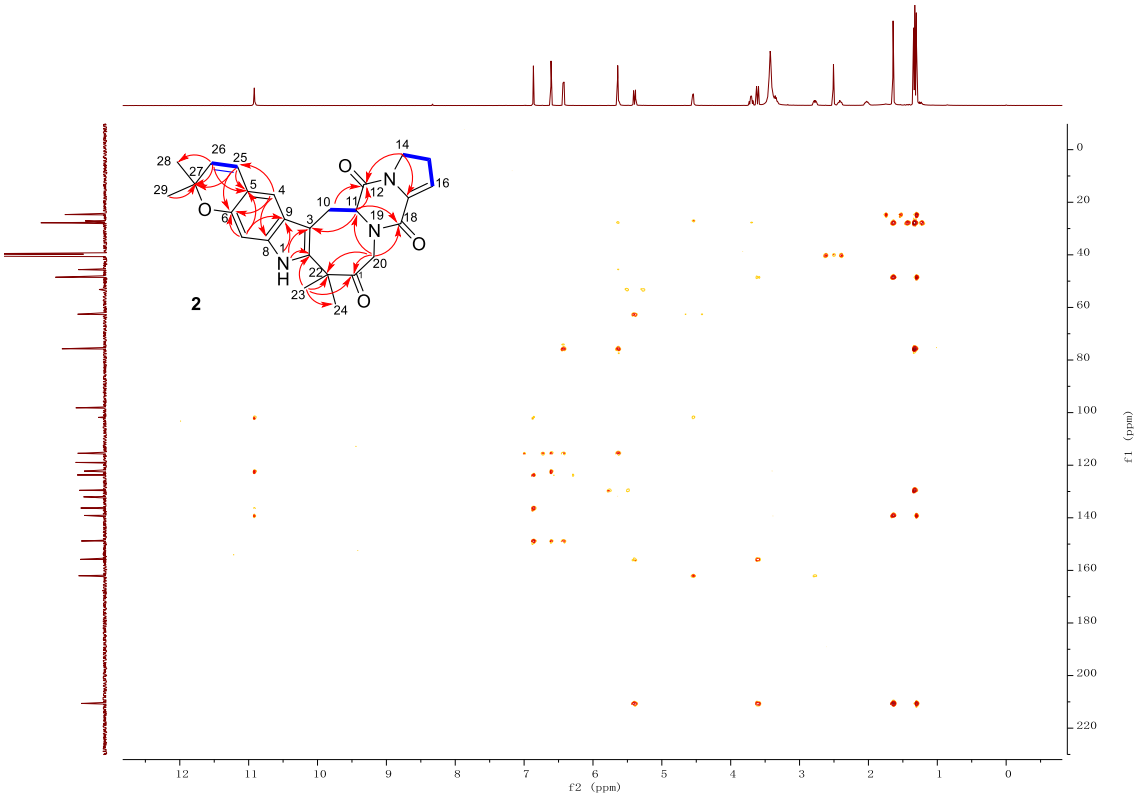


Figure S26: HMBC spectrum of **2** in DMSO-d_6

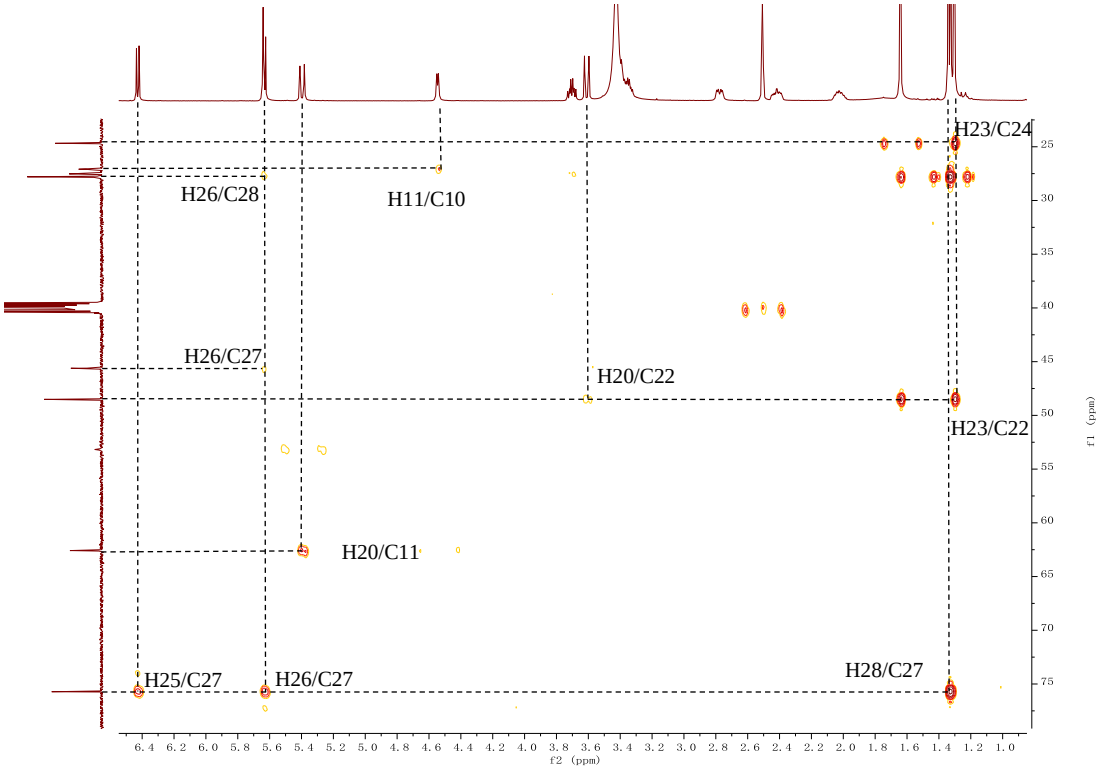


Figure S27: HMBC spectrum of **2** in DMSO-d_6 (From δ_C 25 ppm to δ_C 75 ppm)

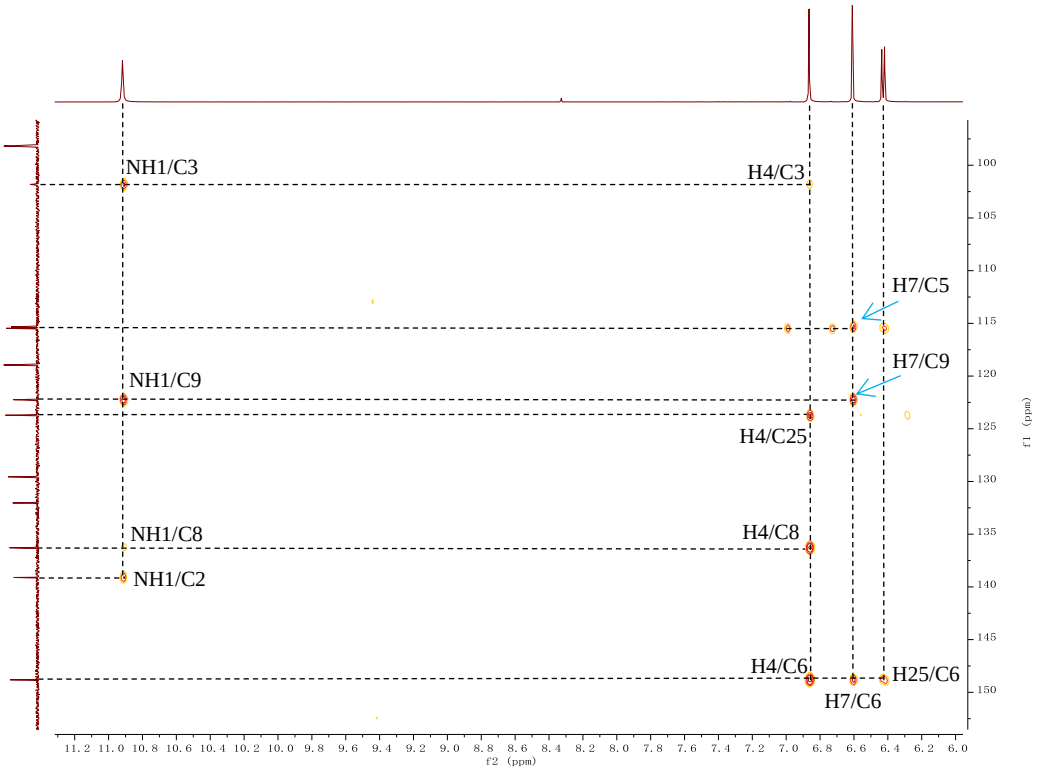


Figure S28: HMBC spectrum of **2** in DMSO-d_6 (From δ_C 100 ppm to δ_C 150 ppm)

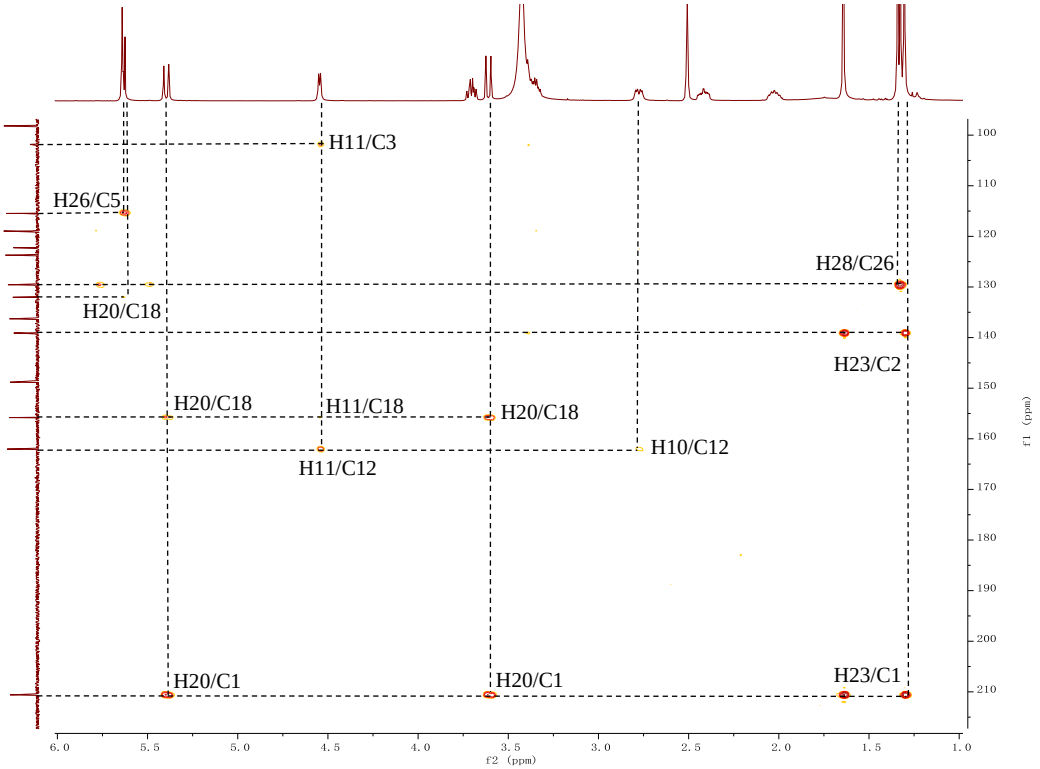


Figure S29: HMBC spectrum of **2** in DMSO-d_6 (From δ_C 100 ppm to δ_C 210 ppm)

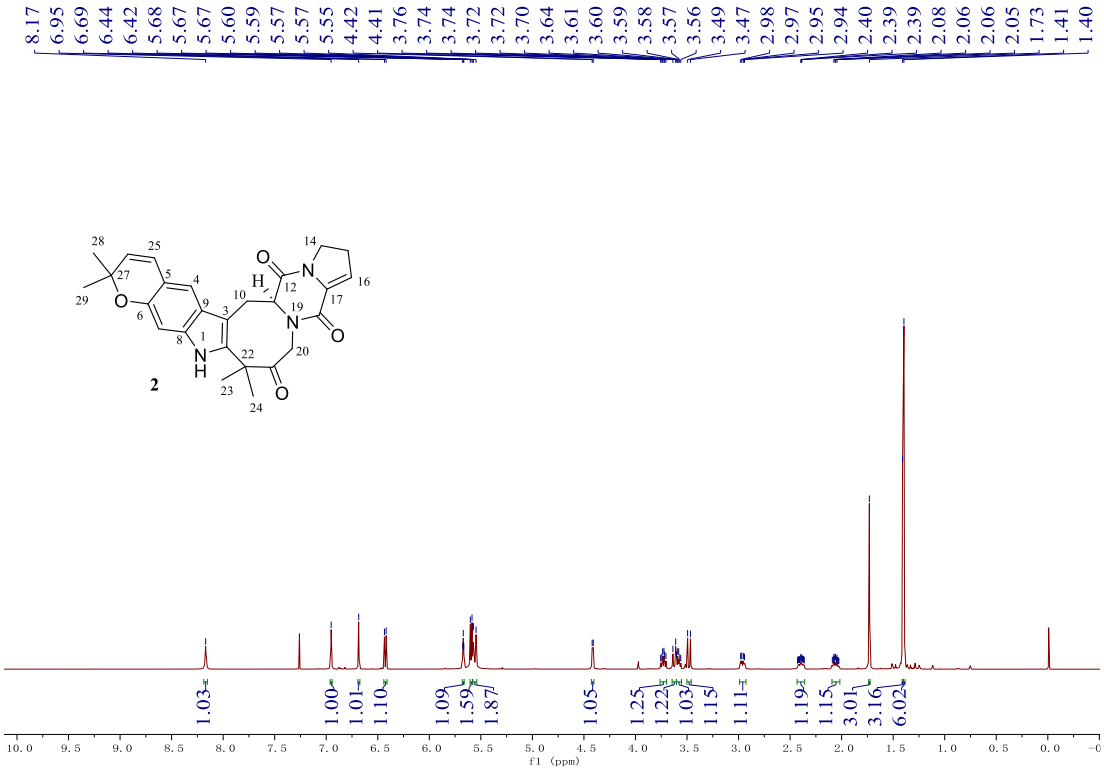


Figure S30: ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **2**

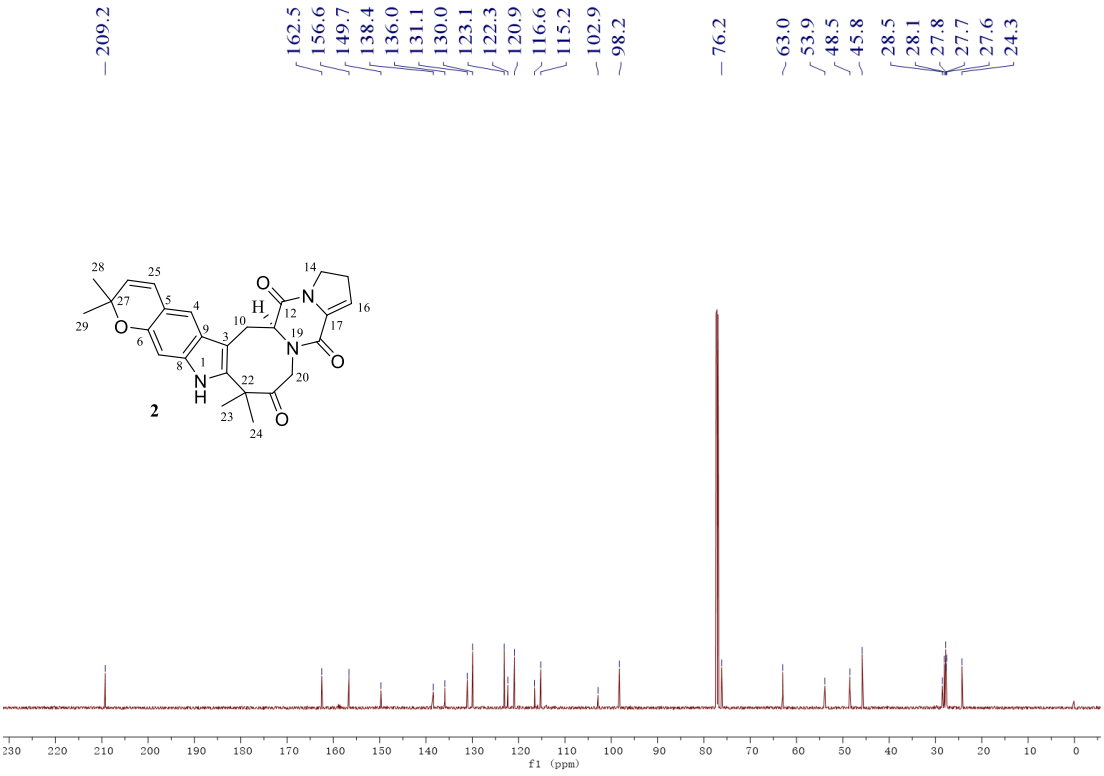


Figure S31: ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of **2**

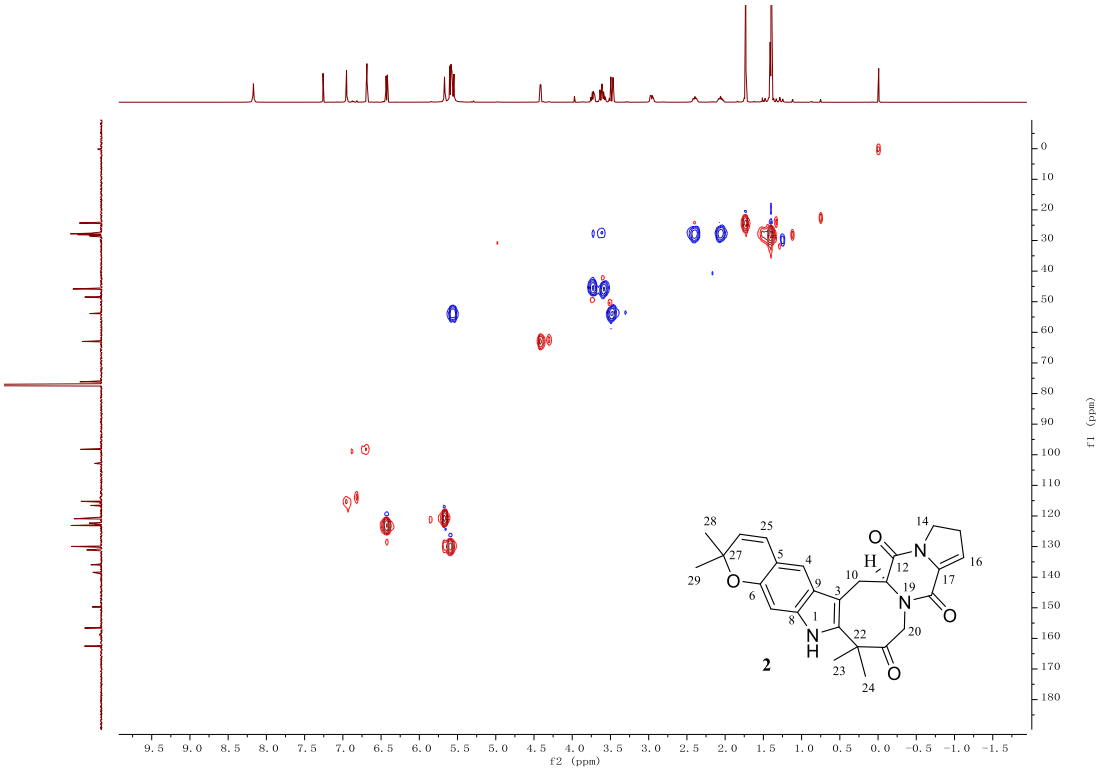


Figure S32: HSQC spectrum of **2** in $CDCl_3$

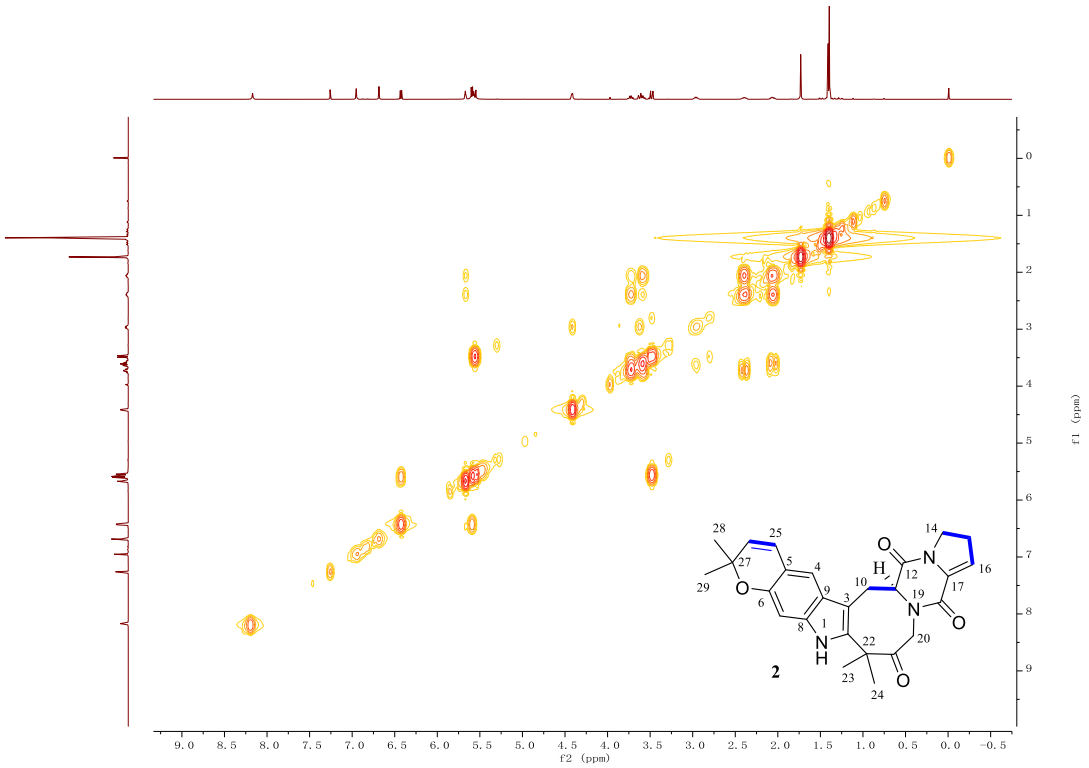


Figure S33: ^{1}H-^{1}H COSY spectrum of **2** in $CDCl_3$

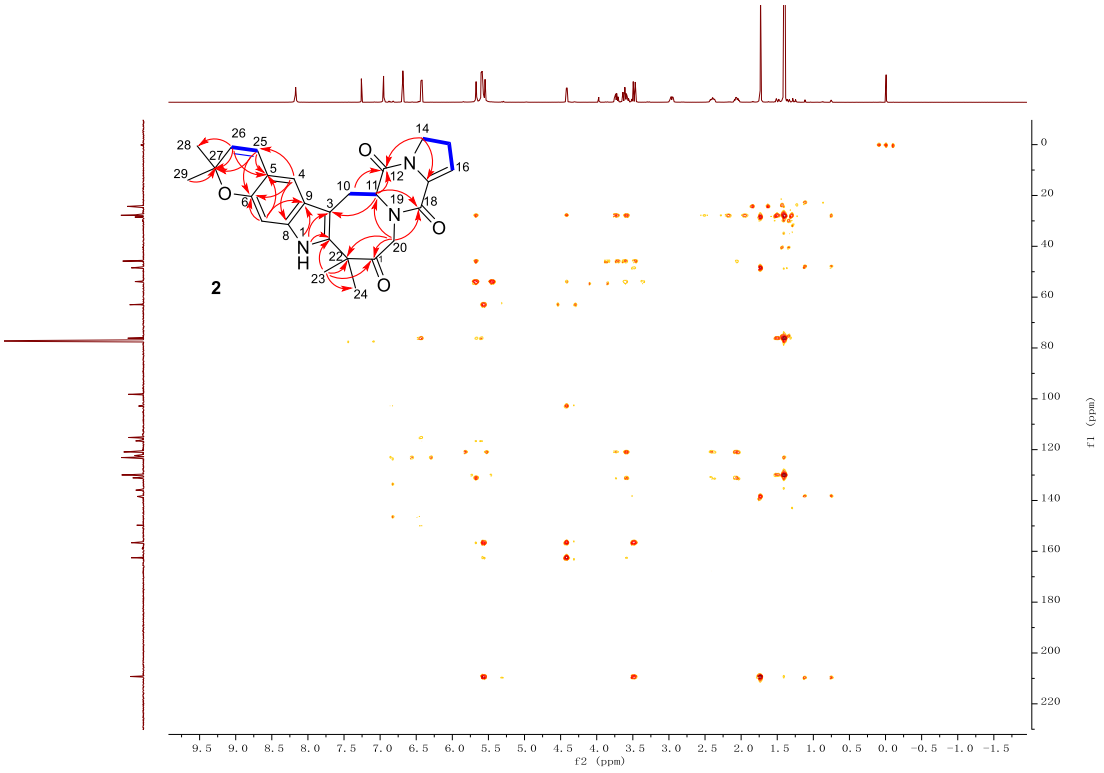


Figure S34: HMBC spectrum of **2** in $CDCl_3$

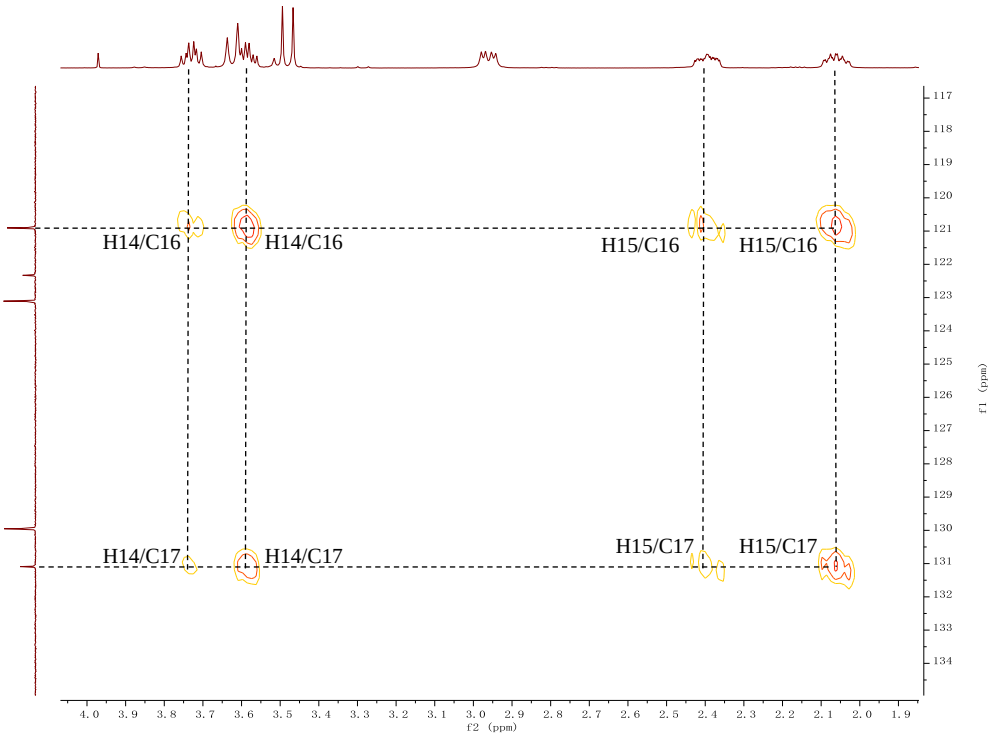


Figure S35: HMBC spectrum of **2** in $CDCl_3$ (From δ_C 117 ppm to δ_C 134 ppm)

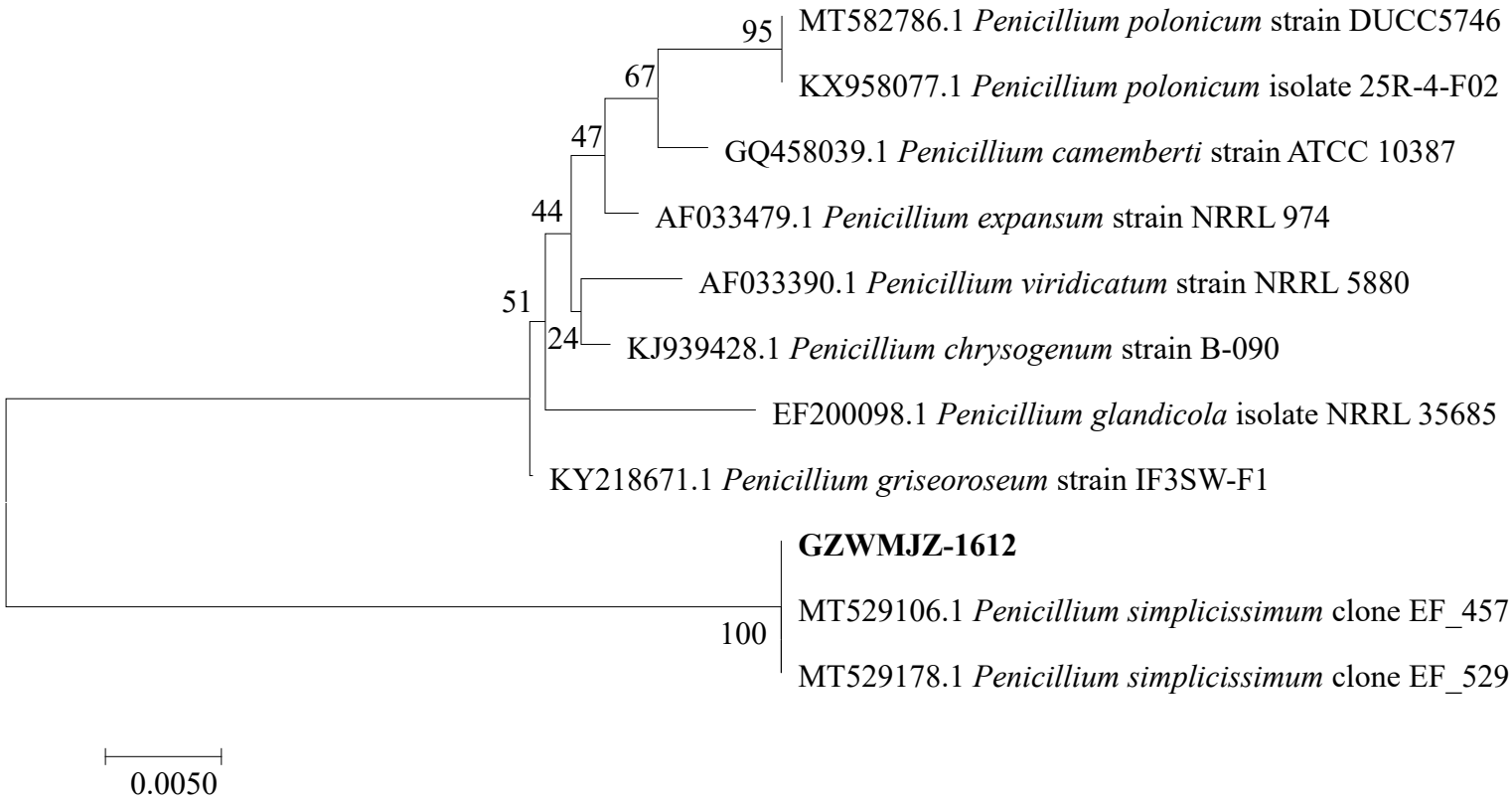


Figure S36: Phylogenetic tree of *Penicillium simplicissimum* GZWMJZ-1612

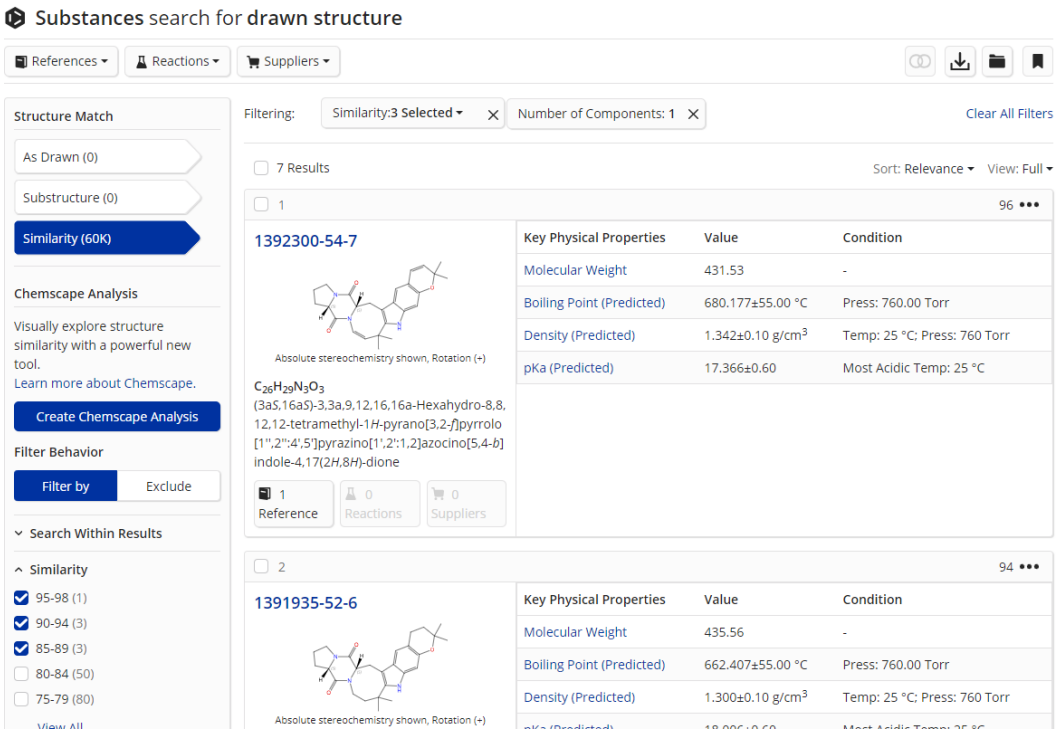


Figure S37: SciFinder report for 1

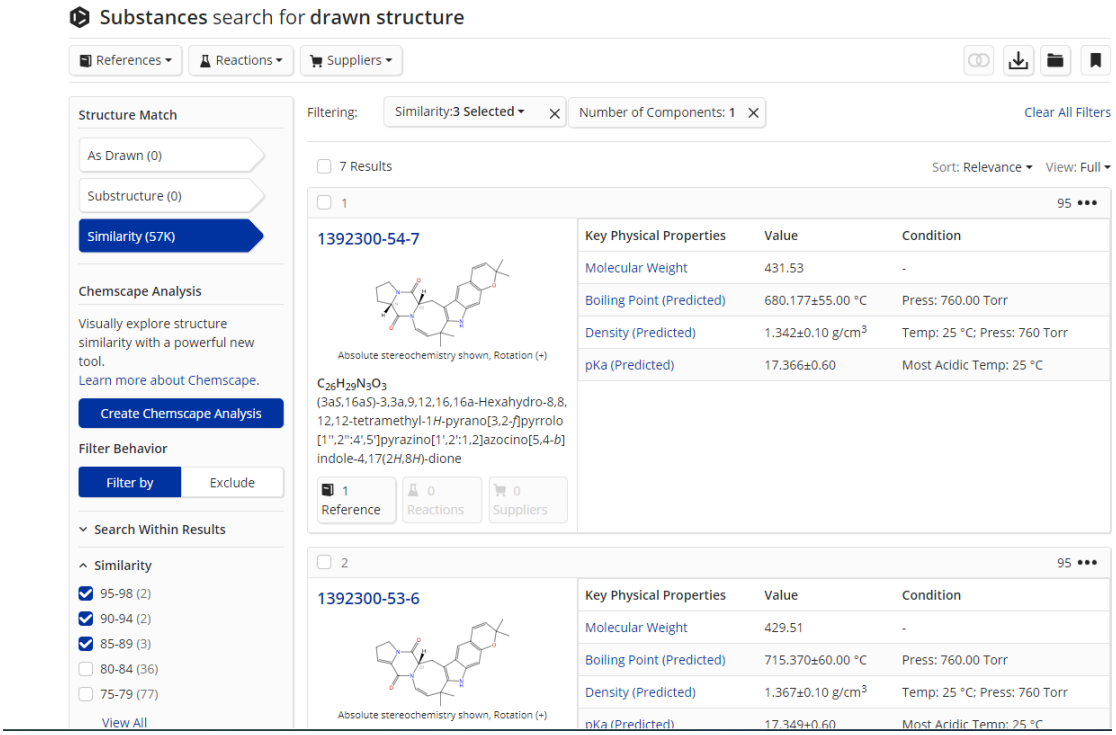


Figure S38: SciFinder report for 2

Apparatus and Reagents

Optical rotations were measured on an AUTOPOL1 polarimeter. UV absorbances were measured on a Beckman DU 640 spectrophotometer. IR spectra were measured as a thin film on a KBr disk using an Anton Paar MCP5100 spectrophotometer. ^{1}H, ^{13}C, HSQC, HMBC, COSY, and NOESY NMR spectra were carried out on a Bruker-600MHz spectrometer with tetramethylsilane as the internal standard. HRESIMS data were obtained using a Waters Xevo TQS and Agilent Technologies 6530 Accurate-MassQ-TOF LC/MS. A binary gradient Hitachi Primaide HPLC system with an evaporative light scattering detector and a 1430 diode array detector were used for purifications with an ODS column (YMC-Pack ODS-A, 5 μm, 250 × 10 mm). TLC was performed on plates precoated with silica gel GF_{254} (10 – 40 μm).

Marfey's method[s1]

This experiment was conducted according to the marfey's method as reported in the literature. compound **1** (0.5 mg) and 1 mL of 6 M HCl was mixed and sealed into a 2 mL pressure-resistant bottle. The mixture was stirred and reacted at 110 °C in an oil bath for 12 h to complete the hydrolysis. After cooling to room temperature, the reaction solution was taken out and evaporated to dryness. The dried mixture was dissolved in 100 μL of ddH_2O, and 40 μL of 1 M $NaHCO_3$ and 100 μL of 25 mM Nα-(5-fluoro-2,4-dinitrophenyl)-L-leucinamide (L-FDLA) acetone solution were added. The reaction was placed in a water bath at 40 °C for 1 hour with no light. Then 20 μL of 2 M HCl was added to terminate the reaction, and then taken out and evaporated to dryness. L-proline and D-proline were separately derivatized with L-FDLA reagent in the same procedure. Finally, the derivatives of compound 1, L-Pro and D-Pro were analyzed by HPLC analysis with C18 column (YMC-Pack ODS-A, 4.6 × 250 mm, 5 μm, 1 mL/min) with linear gradient of acetonitrile MeCN (A) - 0.5‰ trifluoroacetic acid H_2O (B), The gradient was 0 - 40 min 30 - 60% (A), 40 - 45 min 100% (A). The retention times of compound **1**, L-Pro and D-Pro derivatives were 19.4 min, 19.4 min and 22.9 min, respectively.

Oxygen radical absorbance capacity (ORAC) assay[s2]

The anti-oxidative activity of compounds was evaluated by ORAC assay that was carried out mainly by using 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH, 153.0 μM), fluorescein (FL, 81.6 nM), testing compounds, and trolox as a positive control, all of which were dissolved in phosphate buffer solution (PBS, 75 mM, pH 7.4). The concentrations were 6.25 μM for all the tested compounds. In short, each 25 μL of testing compounds, blank (PBS), negative (PBS) and trolox, and 150 μL of FL were added in each well and incubated at 37 oC for 10 min. Each 25 μL of AAPH was then added to the testing compounds, blank and trolox groups, and 25 μL of PBS was added to the negative group. Fluorescence intensity of each well was measured one time every one min for 90 cycles using a Fluoroskan Ascent FL plate-reader (Thermo Scientific Varioskan LUX) at excitation of λ 485 nm and emission of λ 530 nm. The relative fluorescence intensity f was equaled to the ratio of the absolute fluorescence reading to the initial fluorescence reading, and the net area under curve (AUC) was obtained by subtracting the AUC of the blank from that of the compound. The AUC was calculated as $0.5 + f1 + ... fi + ... + f89 + 0.5 \times f90$, in which fi means the ratio of fluorescence reading at time i to the initial fluorescence reading. The final ORAC values were calculated as micromole per liter of trolox equivalents per micromole per liter of the compound (μM TE/μM) by using a regression equation between the trolox concentration and the net area under the FL decay curve. That is, the relative ORAC value = $(AUC_{compound} - AUC_{blank}) / (AUC_{trolox} - AUC_{blank})$.

DPPH radical-scavenging assay [s2,s3]

The experiment was set to five groups, that is blank (methanol, MeOH), sample (mix compound and DPPH solution), background (pure compound solution), negative (pure DPPH solution) and positive (*L*-ascorbic acid (VC) and DPPH solution) controls. DPPH (0.15 mM), compounds (1–100 μM) and VC (1–100 μM) in the following procedures all were dissolved in MeOH. Each 160 μL of MeOH was placed

in negative control and blank groups, while each 160 μL of testing compounds or VC was placed in sample and background groups. Then, MeOH (each 40 μL) was respectively added to blank and background groups, while 40 μL of DPPH was respectively added to negative, positive and sample controls. After 30 min incubation in the dark at rt, the decrease in DPPH radical concentration was monitored by measuring the absorbance at λ 517 nm with a microplate reader (Multiscan Spectrum, Thermo Scientific Varioskan LUX). The DPPH radical-scavenging rate was calculated as:

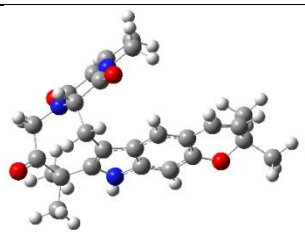
Scavenging rate (%) = [(Anegative – Ablank) – (Asample – Abackground)] / (Anegative – Ablank) × 100%

The IC_{50} (half maximal inhibitory concentration) values of compounds and VC were calculated by SPSS (Statistical Package for the Social Sciences) software from the radical scavenging rates at the final concentrations of 100, 50, 10, 5, and 1 μM.

α-glucosidase inhibitory activity assay[s4]

The inhibitory activities of compounds **1**-**2** against α-glucosidase derived from *Saccharomyces cerevisiae* were evaluated using a previously reported method. The testing compounds were initially dissolved in dimethyl sulfoxide (DMSO) to create a stock solution at a concentration of 10 mM, which was then diluted in phosphate buffer solution (PBS, pH 6.8). The α-glucosidase enzyme (2.0 U/mL, Sigma), 4-nitrophenyl-α-D-glucopyranoside (PNPG, 2.5 mM, Macklin), sodium carbonate (Na_2CO_3, 0.2 M), and acarbose (2.5 mg/mL, Sigma) were directly dissolved in PBS for the assay. In a 96-well microplate, 20 μL of the compound solutions and acarbose were mixed with 20 μL of α-glucosidase and 60 μL of PBS, serving as the drug and positive control groups, respectively. Pure PBS solution was utilized as the blank group. Following a 15-minute incubation at 37 °C, 20 μL of PNPG solution was added to each well in the testing groups, and the mixtures were further incubated at 37 °C for an additional 30 minutes. To terminate the reaction, 80 μL of Na_2CO_3 solution was subsequently added to each well. The absorbance was then measured using a microplate reader (Multiscan Spectrum, Thermo Scientific Varioskan LUX) at a wavelength of 405 nm. The inhibitory rate was calculated as $[1 - (A_{drug}/A_{blank})] \times 100\%$. The IC_{50} values for the compounds were determined using SPSS software, based on the inhibitory rates recorded at final concentrations of 500, 250, 50, 25, 5, 1, and 0.2 μM.

·Stable conformers for ECD calculation of compound 2

No.	Energy (kcal/mol)	Conformers	Boltzmmann distribution
2	-1472.193793		100%

References

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