Supporting Information

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Three New Polyketides from the Insect-Associated Fungus *Letendraea* sp. 5XNZ4-2

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1. MTPA Esters of Compound 1

***4, 7, 10-tri-S-MTPA Ester of 1 (1a)*:** White amorphous solid. ^{1}H NMR (600 MHz, in $CDCl_3$) δ_H 7.24−7.52 (15H, m, MTPA-Ar), 5.52 (1H, brs, H-7), 5.48 (1H, brs, H-4), 5.26 (1H, ddd, *J* = 9.2, 3.6, 2.0 Hz, H-10), 4.95 (1H, m, H-9), 4.59 (1H, dd, *J* = 13.6, 3.6 Hz, H-2a), 4.54 (1H, ddd, *J* = 13.6, 6.0, 2.0 Hz, H-2b), 3.51 (3H, brs, MTPA-OMe), 3.46 (3H, brs, MTPA-OMe), 3.45 (3H, brs, MTPA-OMe), 1.93 (1H, m, H-6a), 1.87(1H, m, H-5a), 1.83 (1H, m, H-6b), 1.77 (1H, m, H-5b), 1.70 (1H, m, H-11a), 1.40 (1H, m, H-12a), 1.35 (1H, m, H-11b), 1.30 (1H, m, H-12b), 0.87 (3H, t, *J* = 7.2 Hz, H-13).

***4, 7, 10-tri-R-MTPA Ester of 1 (1b*):** White amorphous solid. ^{1}H NMR (600 MHz, in $CDCl_3$) δ_H 7.25−7.52 (15H, m, MTPA-Ar), 5.58 (2H, brs, H-7, H-4), 5.21 (1H, m, H-10), 4.87 (1H, m, H-9), 4.57 (1H, dd, *J* = 13.7, 3.3 Hz, H-2a), 4.46 (1H, ddd, *J* = 13.7, 6.1, 2.0 Hz, H-2b), 3.53 (3H, brs, MTPA-OMe), 3.50 (3H, brs, MTPA-OMe), 3.47 (3H, brs, MTPA-OMe), 2.13 (1H, m, H-6a), 2.11 (1H, m, H-5a), 1.96 (1H, m, H-6b), 1.94 (1H, m, H-5b), 1.54 (1H, m, H-11a), 1.20 (1H, m, H-11b), 1.15 (1H, m, H-12a), 1.10 (1H, m, H-12b), 0.78 (3H, t, *J* = 7.3 Hz, H-13).

2. Cytotoxic Assay

Cytotoxicity of **1** were measured by the MTT assay [1] against the prostate cancer PC3 cell line (more sensitive than other cancer cells). Tumor cell lines were seeded in 96-well plates (4000 per well in 100 μL). After 24 h of incubation cells were treated with gradient concentrations (100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, 3.125 μM) for another 72h. Afterwards, MTT solution (5.0 mg/mL in RPMI-1640 media, Sigma, St. Louis, MO, USA) was added (20 μL/well) and then plates were incubated for another 4 h at 37℃. The compounds were dissolved in DMSO and cell growth inhibition assay was performed as reported previously. Doxorubicin (ADR) was used as a positive control. compound **1** didn't exhibit cytotoxicity against PC_3 cells (the cell growth inhibition rate was 13% at 10 μg/mL).

3. Antibacterial Assay

The antibacterial activities of **1** was evaluated with *S. aureus*, *S. epidermidis*, *E. coli*, *M. albicans* and *P. aeruginosa* using Kirby−Bauer Diffusion method [2] and gentamicin, vancomycin, ampicillin, amphotericin B and gentamicin served as the positive drugs, respectively.

The stock solution of compound **1** was prepared in methanol with a series of concentration (25, 5.0, 1 and 0.2 mg/mL). Positive control drugs gentamicin, vancomycin and ampicillin were dissolved in purified water with the concentration of 500 ug/mL, amphotericin B was dissolved in DMSO with the concentration of 100 ug/mL.

S. aureus, *S. epidermidis*, *E. coli*, *M. albicans* and *P. aeruginosa* were activated in nutrient broth at 37℃ for 6~8 h with 200 rpm and the culture broth was poured on the surface of MEB Agar (20 g MEB and 20 g agar in 1 L water) plates inside the cabinet.

(Make sure to have a uniform distribution of cells on the plates). Label the location of negative control (methanol), the positive drug and different contents of tested compounds (100 µg, 20 µg, 4 µg and 0.8 µg for **1**) on the agar side of the plate and outside the lid. Arrange the discs as to mimic their positions outside the lid and add 4 µL of corresponding concentrations of tested compounds and methanol to the discs. The contents of the positive drugs gentamicin, ampicillin and amphotericin B were 3 ug, 1.5 ug and 0.35 ug, respectively. Transfer the discs from the lid to the agar plate using a sterile forcep. Finally, place the plates in an incubator agar side up at 37ºC for 12 h to observe the inhibition zone.

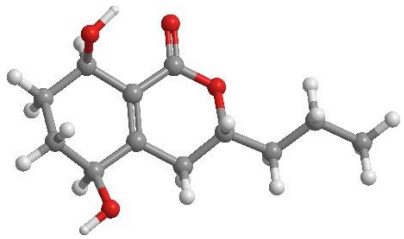
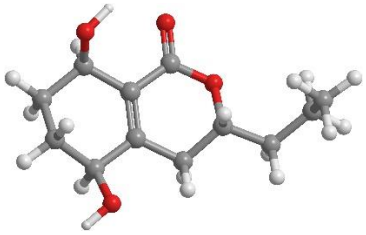
Unfortunately, compound **1** didn't show any inhibition zone to the texted microbials at all the concentrations (100 µg, 20 µg, 4 µg and 0.8 µg/disc).

References

[1] T. Mosmann (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, *J. Immunol. Meth.* **65**, 55-63.

[2] A. W. Bauer, W. M. Kirby，J. C. Sherris and M. Turck (1966). Antibiotic susceptibility testing by a standardized single disk method, Amer. *J. Clin. Pathol.* **45**, 493:496.

Table S1. Optimized Cartesian Coordinates for the lowest energy conformers reoptimized at b3lyp/6-31+g(d,p) level in MeOH for 4*R*, 7*S*, 10*R*-**3** (C1, C2) and 4*R*, 7*S*, 10*S*-**3** (C3, C4).

 C1 74.03% -768.9420838 Hartree				 C2 25.97% -768.9410956 Hartree			
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C	3.24826200	1.08303000	0.31337200	C	3.15369000	0.99560700	0.48894200
C	1.98719400	1.70648100	-0.28798400	C	1.98523300	1.66236200	-0.23936500
C	0.82628600	0.73221600	-0.29078500	C	0.79299300	0.73322800	-0.34957400
C	1.02656400	-0.60306900	-0.23056400	C	0.93765800	-0.60863000	-0.27362700
C	2.39108700	-1.26347100	-0.14107800	C	2.26242900	-1.31925300	-0.05800200
C	-0.56904400	1.27701700	-0.44895400	C	-0.56131000	1.32910600	-0.63132800
C	-1.59976400	0.33357300	0.15021400	C	-1.67506500	0.42226200	-0.13258800
O	-1.37450200	-1.03834300	-0.31878700	O	-1.45859000	-0.95502800	-0.58859600
C	-0.12259400	-1.53542800	-0.32355700	C	-0.23140900	-1.49847800	-0.47420000
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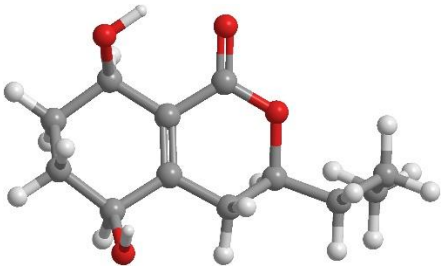
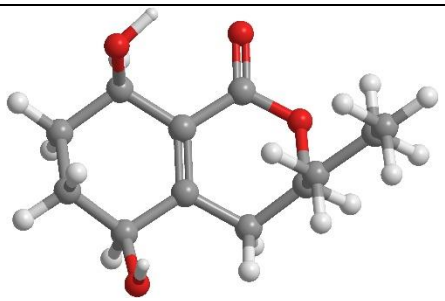


C3 98.61%

-768.9441117 Hartree



C4 1.39%

-768.9400902 Hartree

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C	0.24257900	-1.46268500	-0.32909200	C	0.24257900	-1.46268500	-0.32909200
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H	4.09663400	-1.05253800	-0.47643000	H	4.09663400	-1.05253800	-0.47643000

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H	5.48800300	-0.56332400	1.56098600	H	5.48800300	-0.56332400	1.56098600
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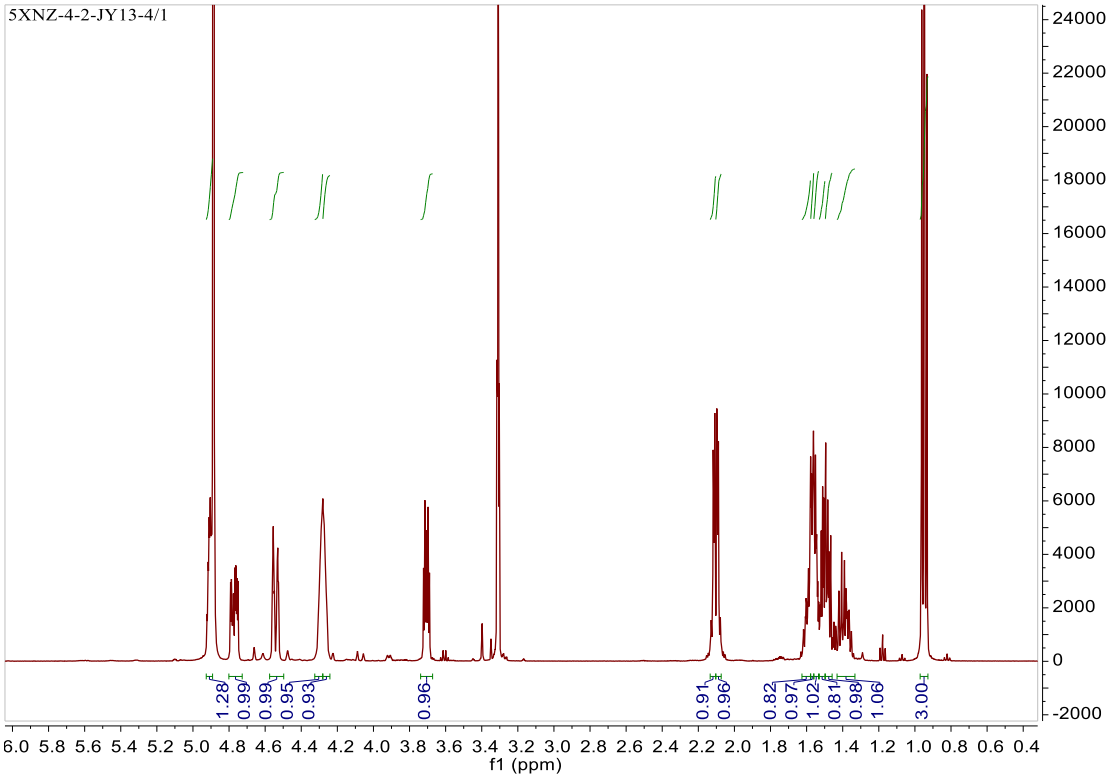

Figure S1: ^{1}H NMR (500 MHz, CD_3OD) spectrum of letendronol D (**1**)

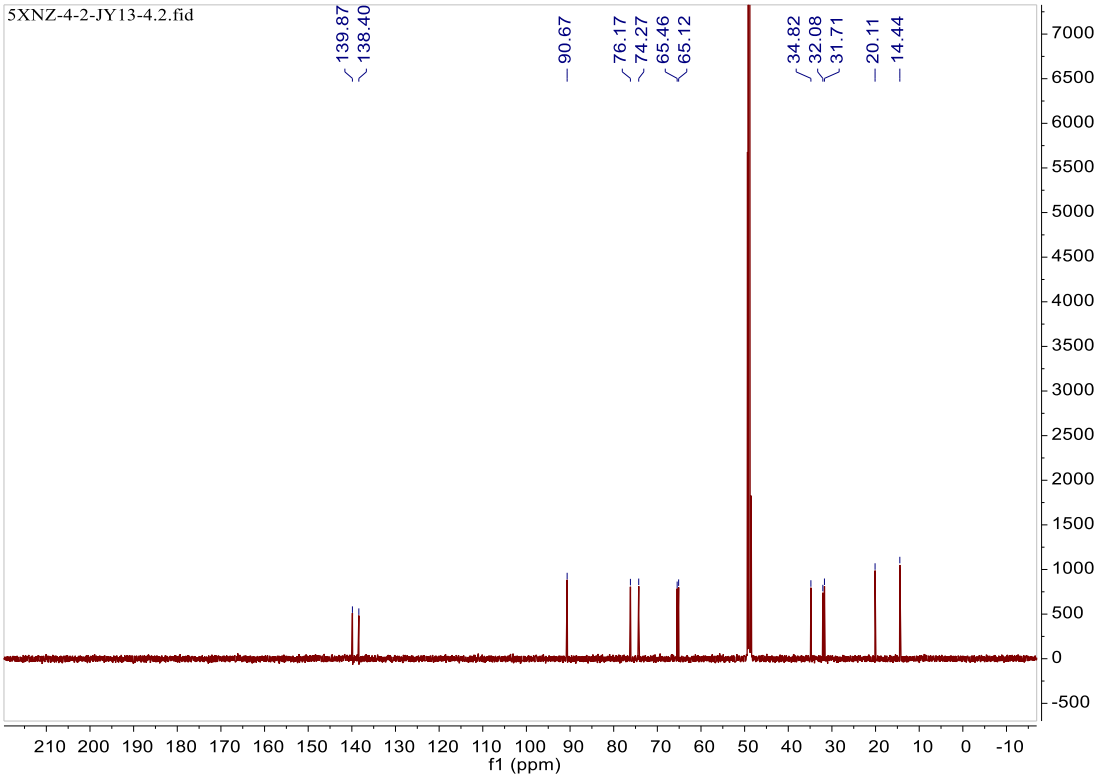

Figure S2: ^{13}C NMR (125 MHz, CD_3OD) spectrum of letendronol D (**1**)

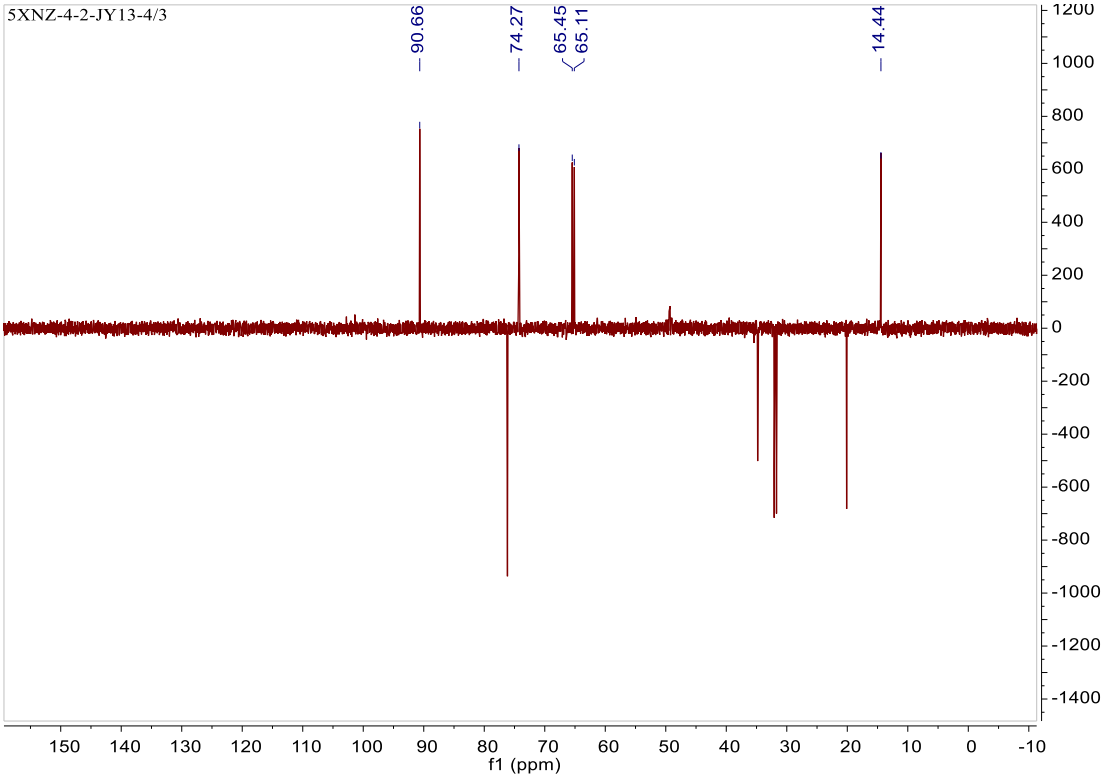

Figure S3: DEPT (CD_3OD) spectrum of letendronol D (**1**)

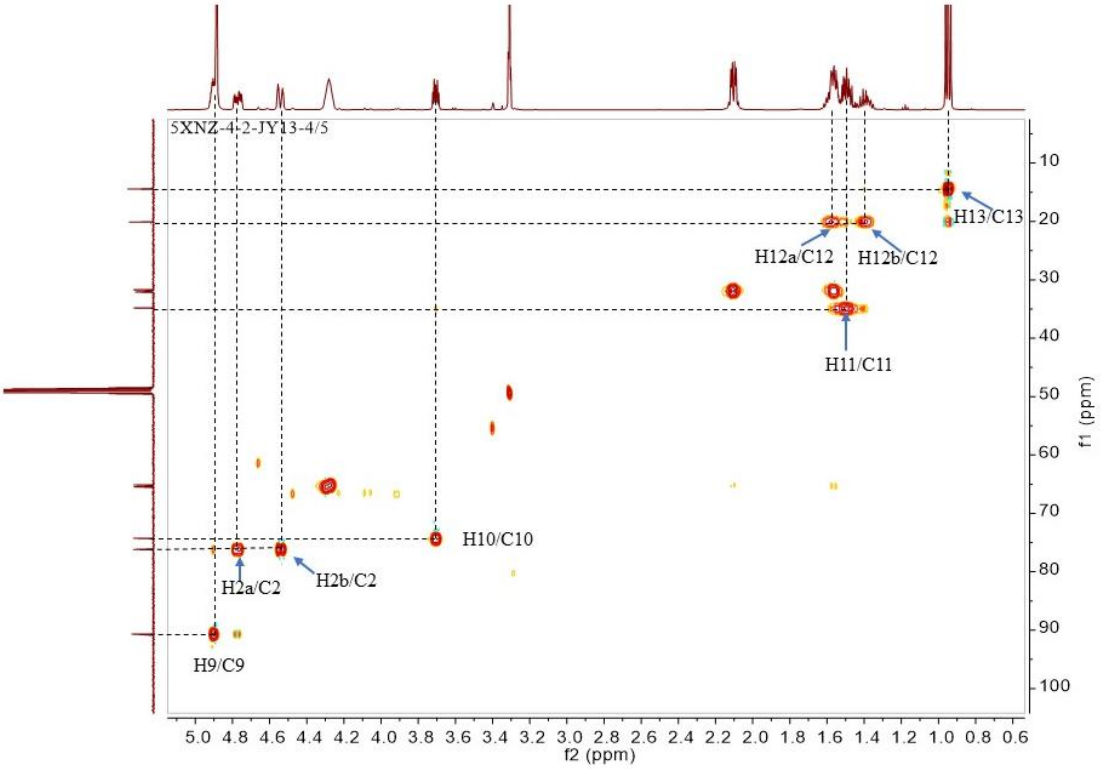

Figure S4: HMQC (CD_3OD) spectrum of letendronol D (**1**)

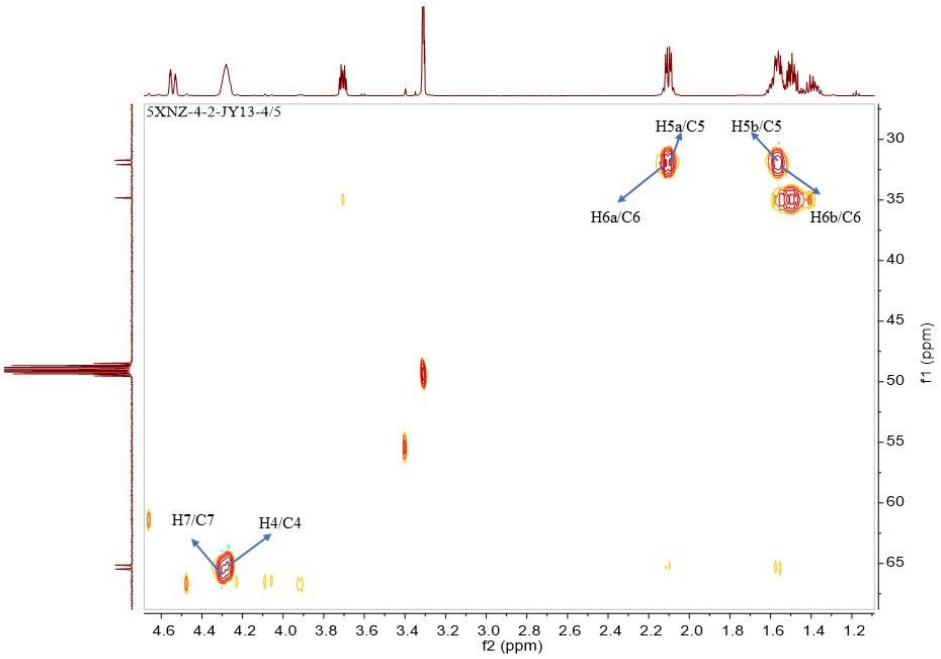

Figure S5: HMQC (CD_3OD) spectrum of letendronol D (**1**) (From δ_C25 ppm to δ_C 70 ppm)

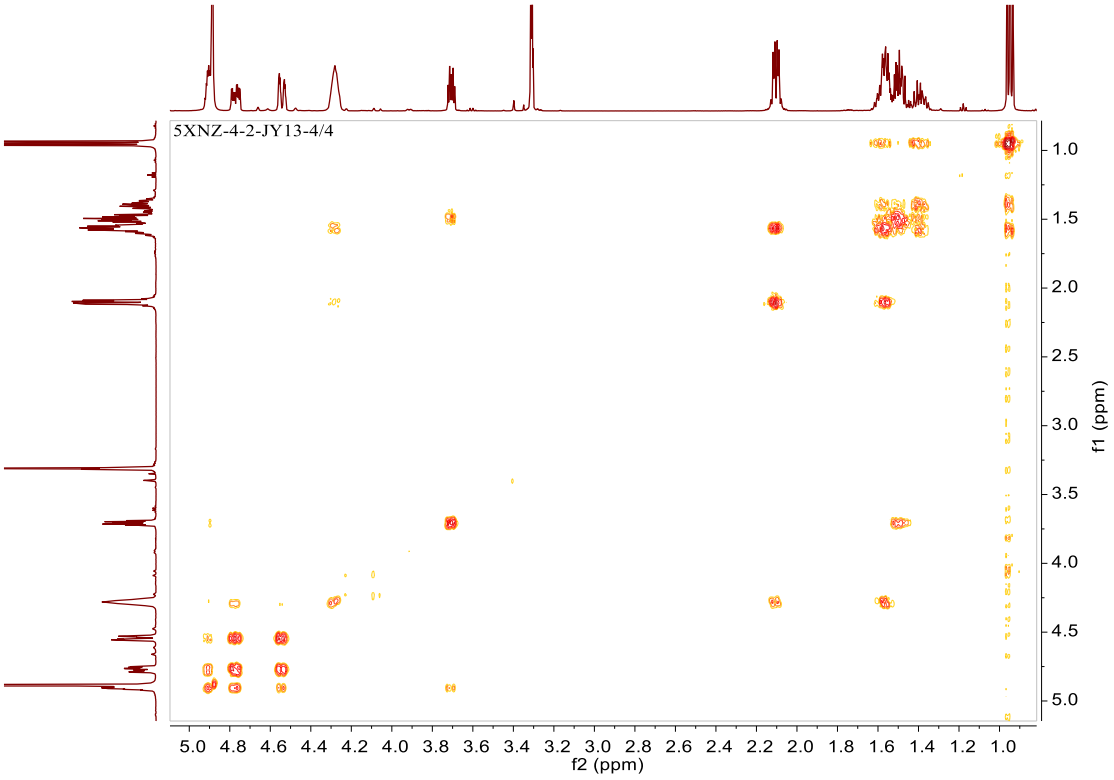

Figure S6: ^{1}H–^{1}H COSY (CD_3OD) spectrum of letendronol D (**1**)

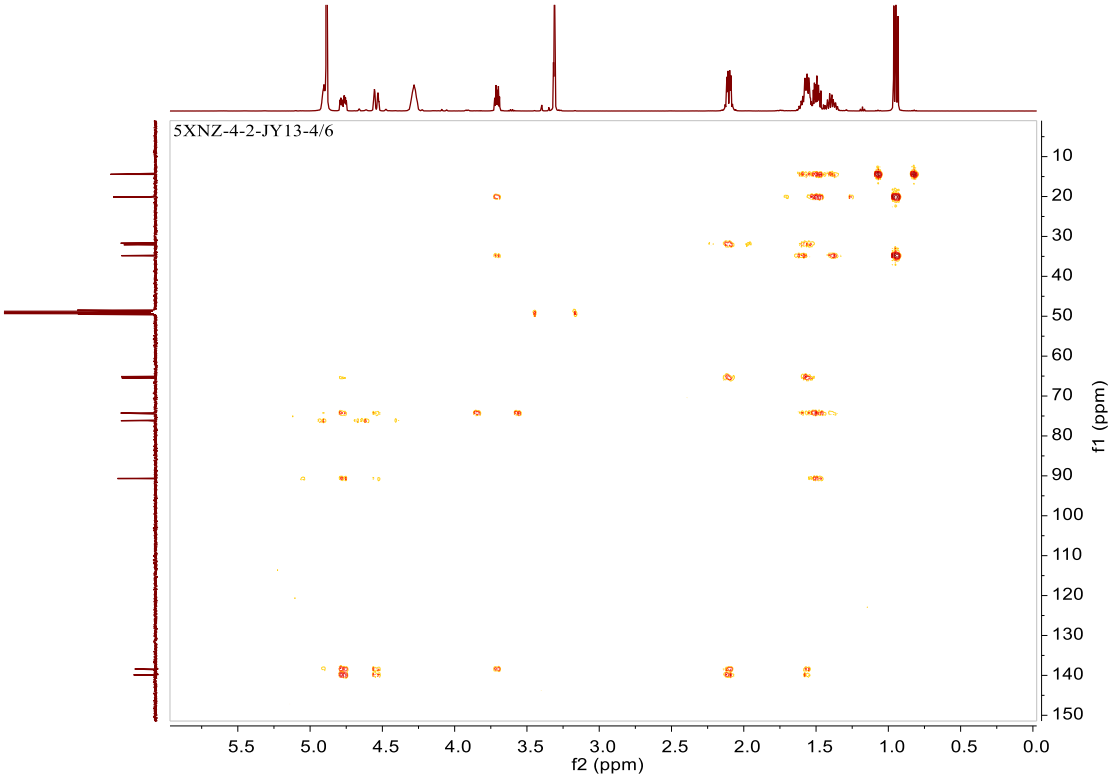

Figure S7: HMBC (CD_3OD) spectrum of letendronol D (**1**)

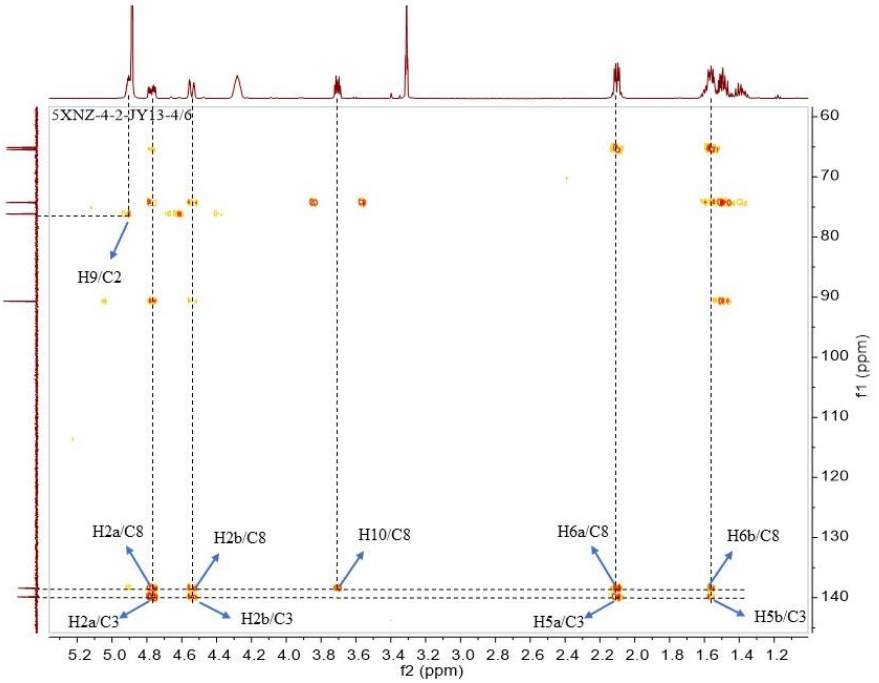

Figure S8: HMBC (CD_3OD) spectrum of letendronol D (**1**) (From δ_C60 ppm to δ_C 145 ppm)

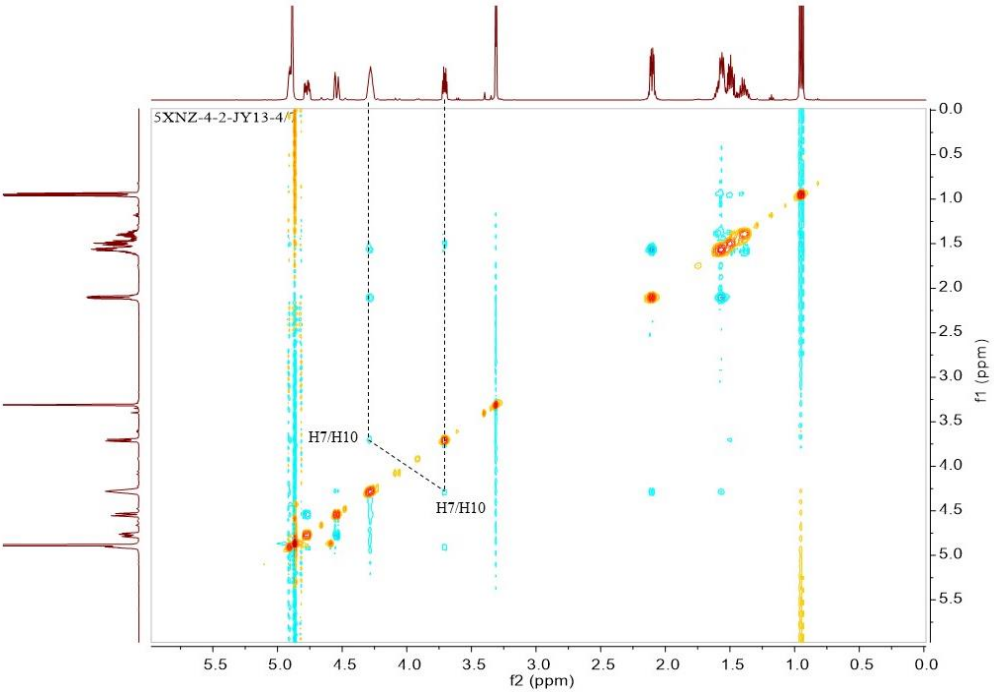

Figure S9: NOESY (CD_3OD) spectrum of letendronol D (**1**)

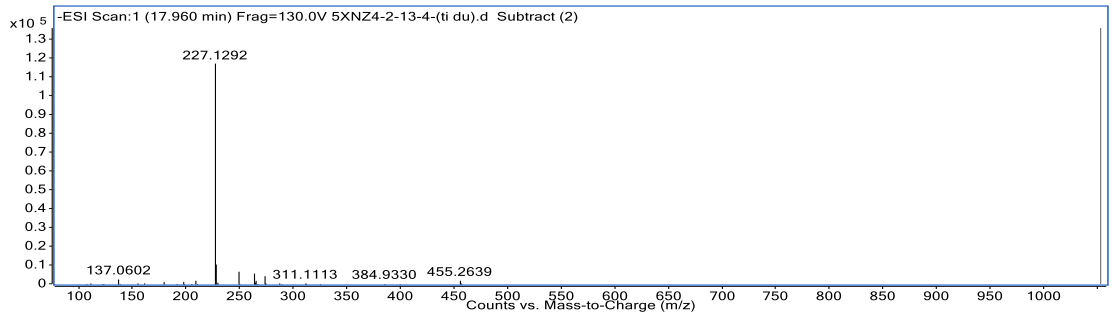

Figure S10: HRESIMS spectrum of letendronol D (**1**)

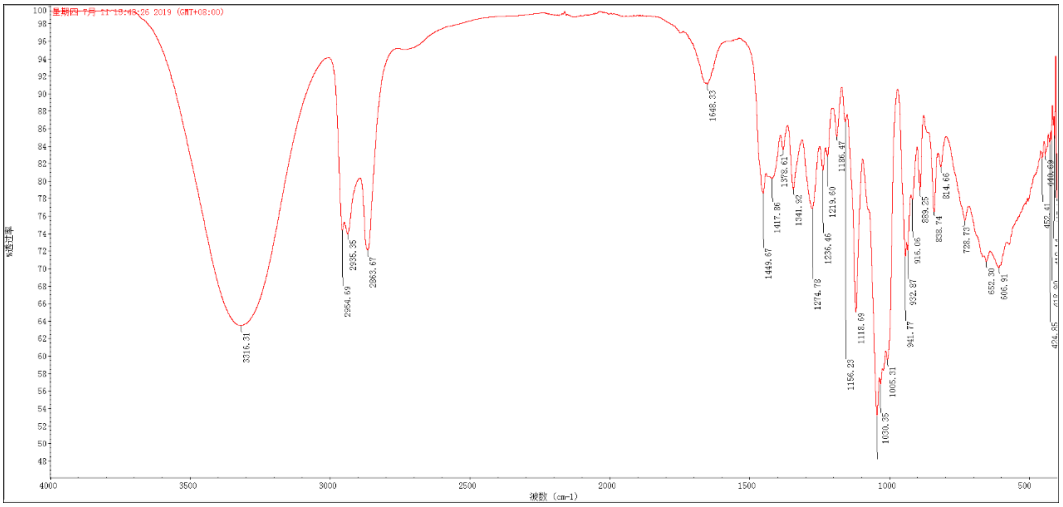

Figure S11: IR spectrum of letendronol D (**1**)

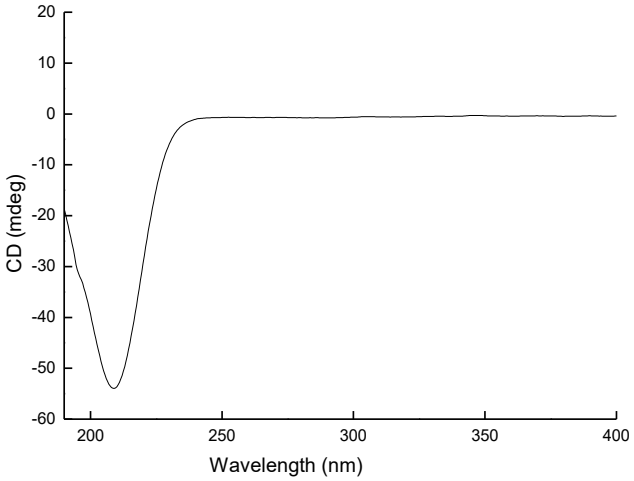

Figure S12: CD spectrum of letendronol D (**1**)

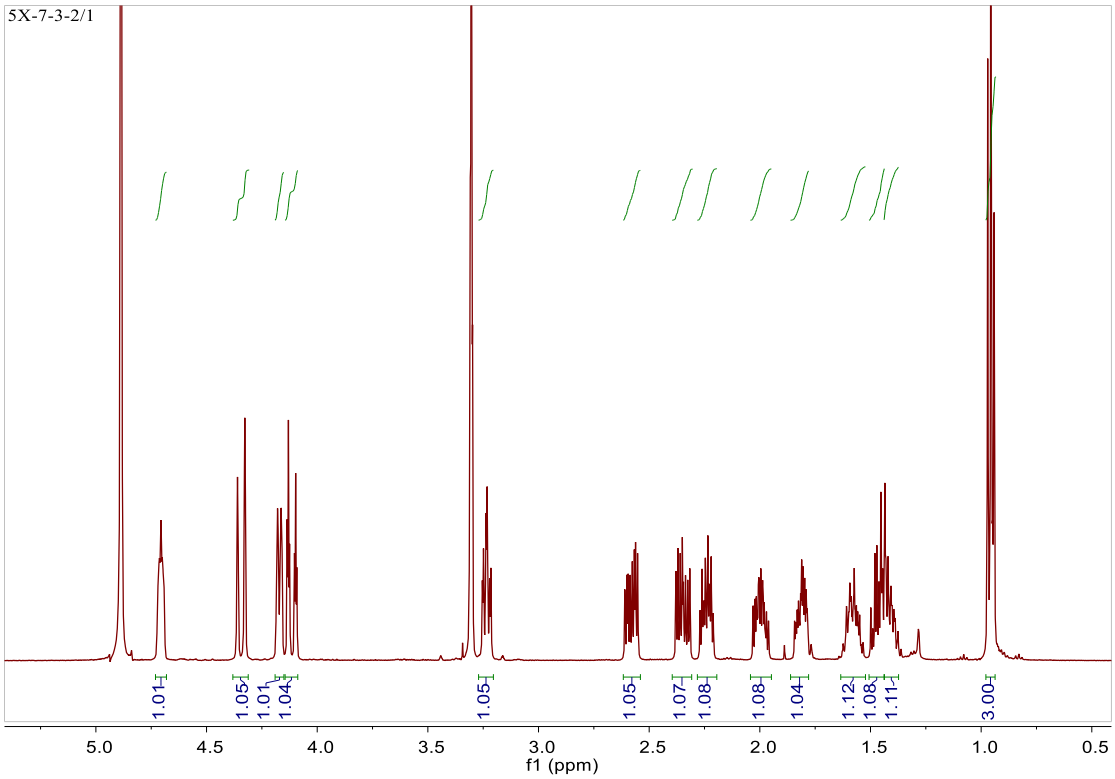

Figure S13: ^{1}H NMR (600 MHz, CD_3OD) spectrum of phomopsiketone H (**2**)

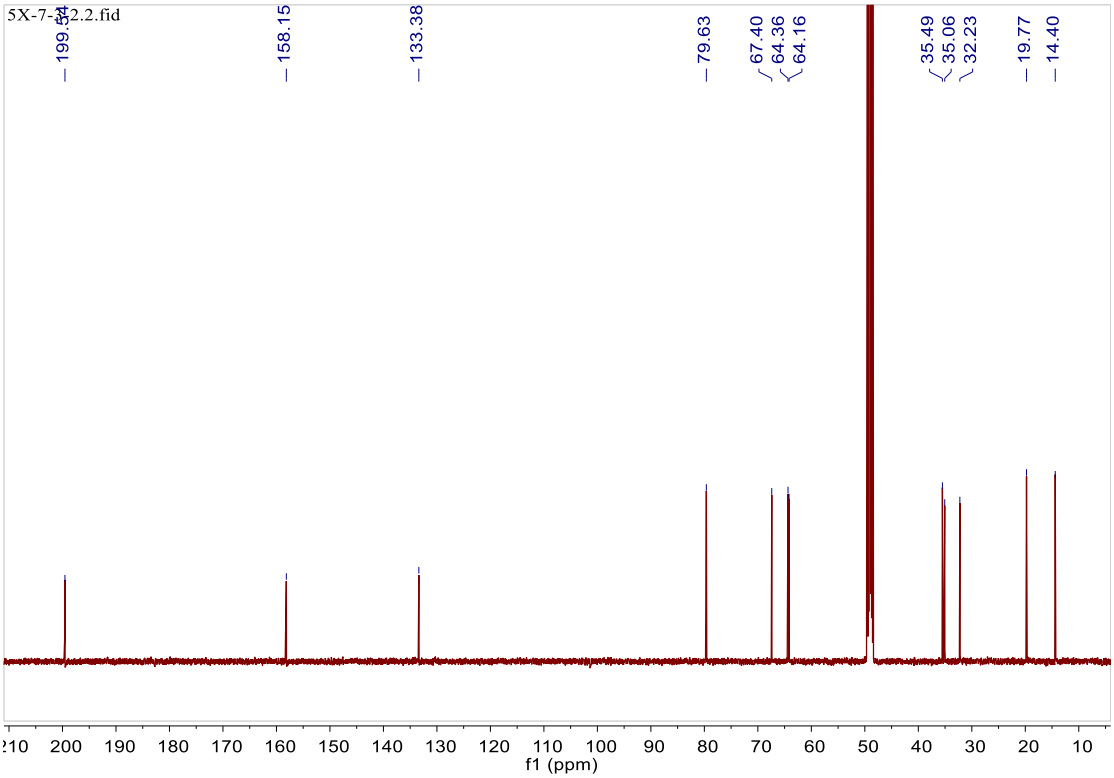

Figure S14: ^{13}C NMR (150 MHz, CD_3OD) spectrum of phomopsiketone H (**2**)

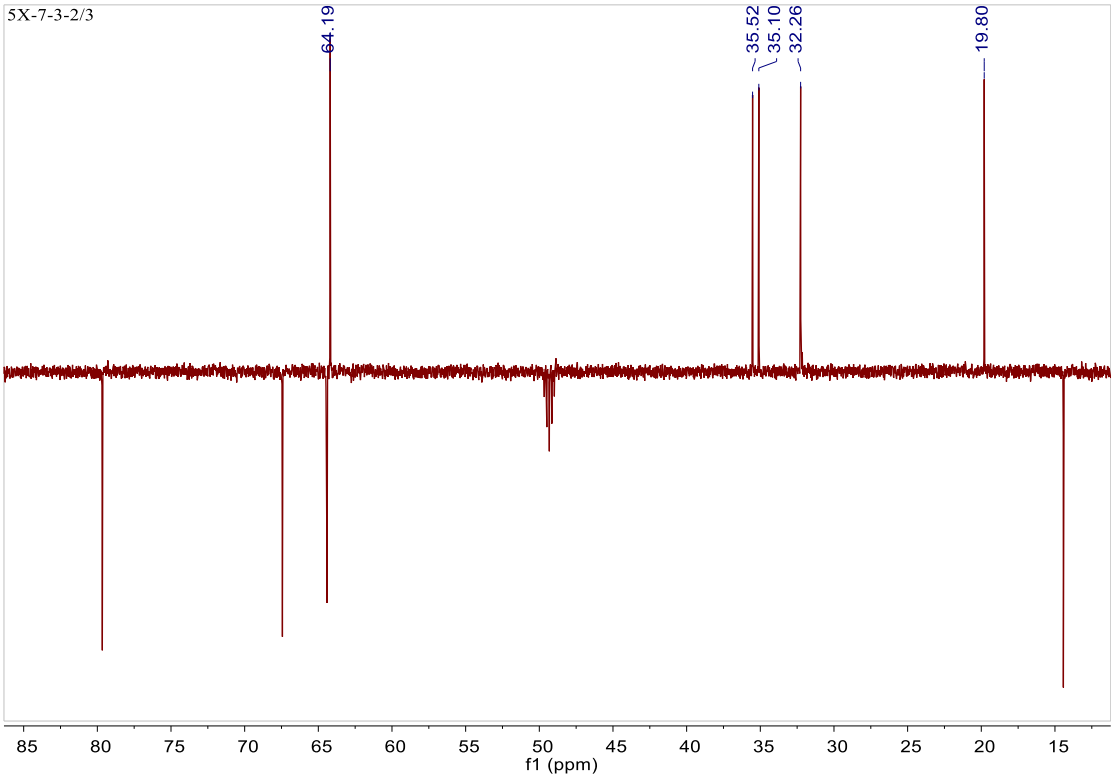

Figure S15: DEPT (CD_3OD) spectrum of phomopsiketone H (**2**)

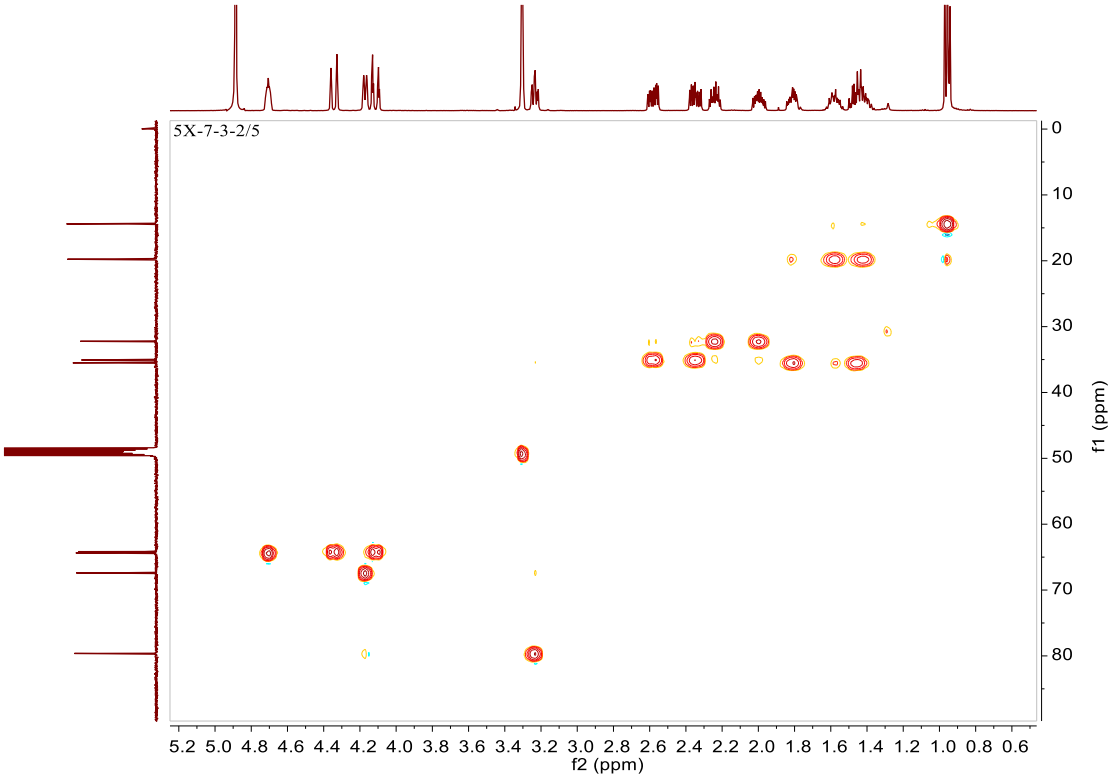

Figure S16: HMQC (CD_3OD) spectrum of phomopsiketone H (**2**)

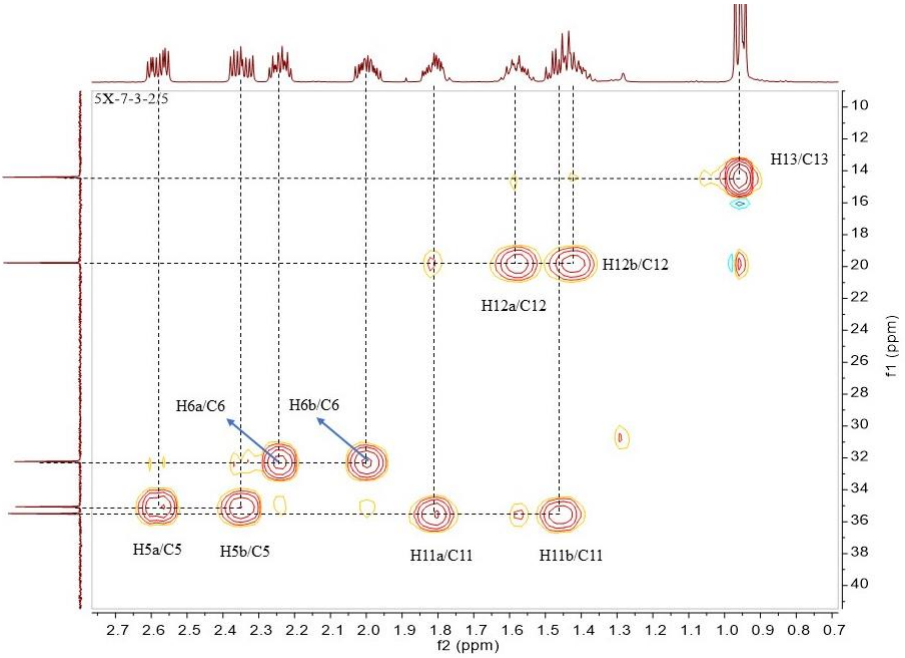

Figure S17: HMQC (CD_3OD) spectrum of phomopsiketone H (**2**) (From δ_C 10 ppm to δ_C 45 ppm)

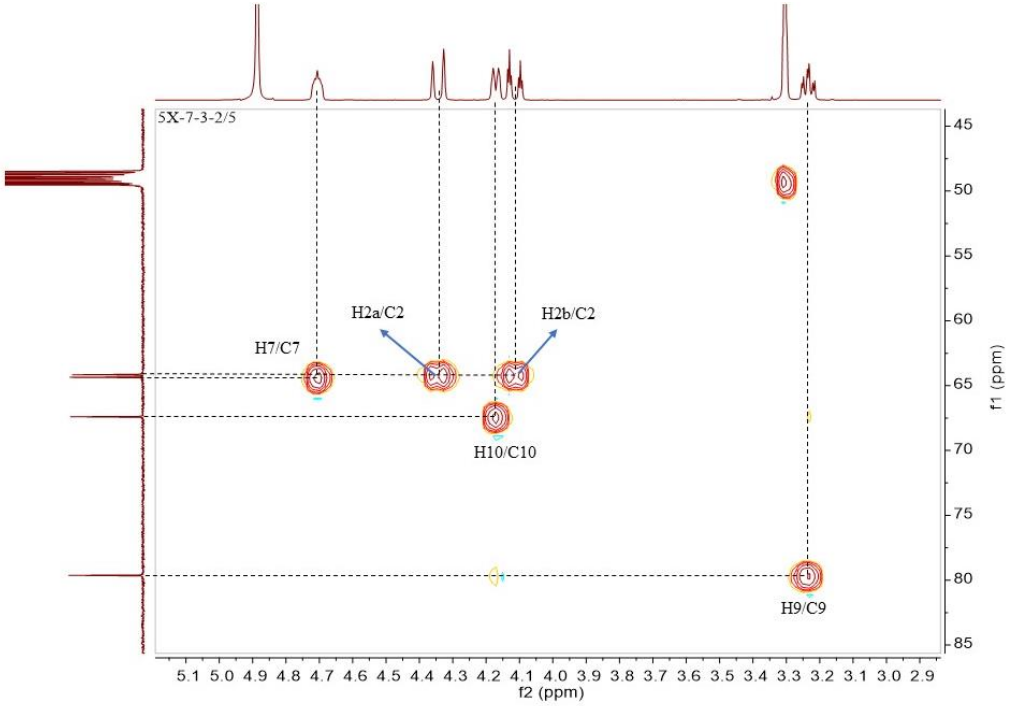

Figure S18: HMQC (CD_3OD) spectrum of phomopsiketone H (**2**) (From δ_C 45 ppm to δ_C 85 ppm)

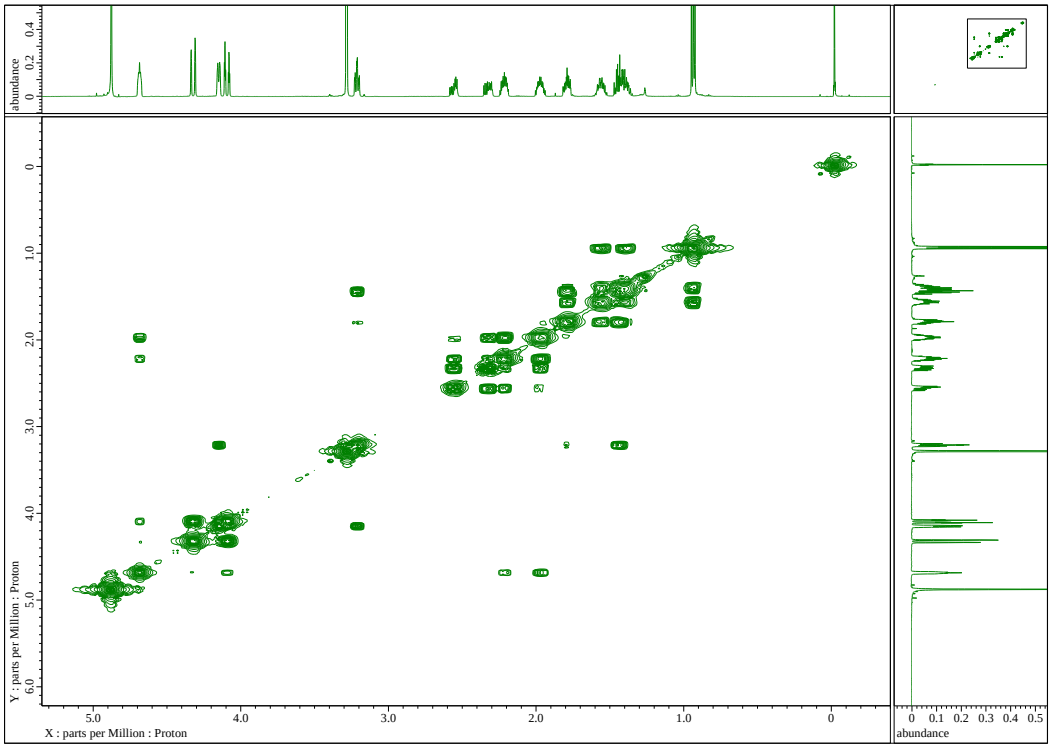

Figure S19: ^1H-^1H COSY (CD_3OD) spectrum of phomopsiketone H (**2**)

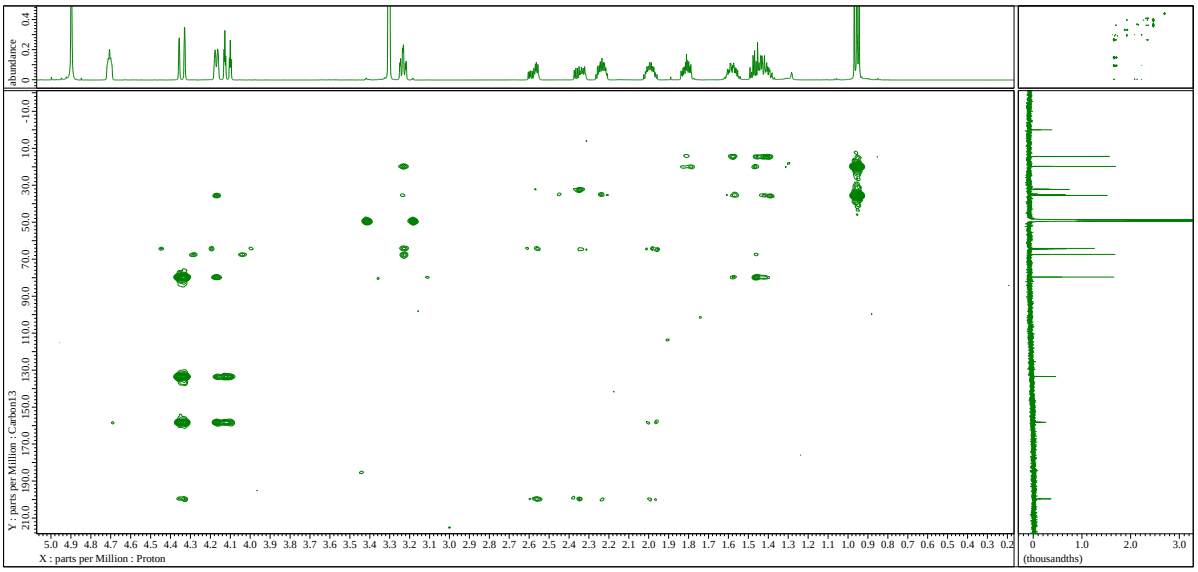

Figure S20: HMBC (CD_3OD) spectrum of phomopsiketone H (**2**)

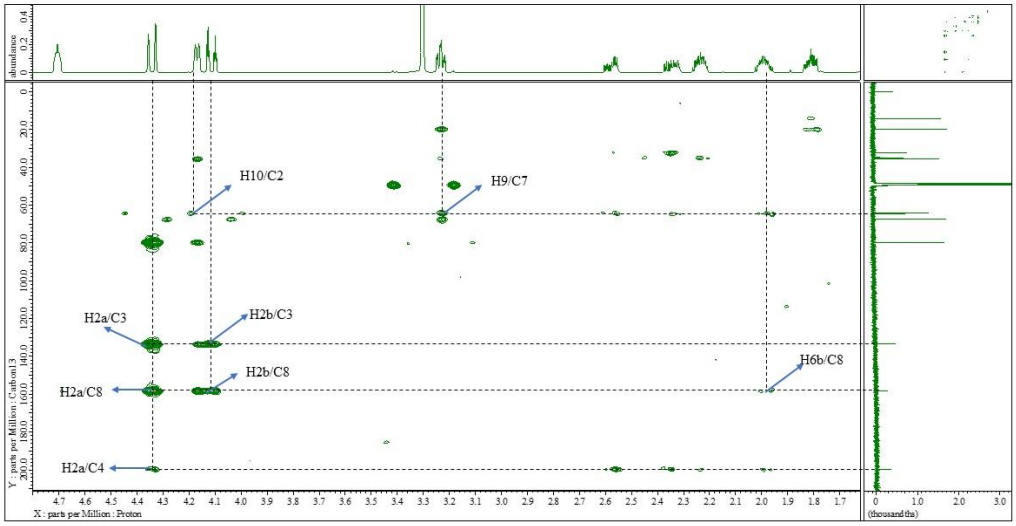

Figure S21: HMBC (CD_3OD) spectrum of phomopsiketone H (**2**) (From δ_H 1.7 ppm to δ_H 4.7 ppm)

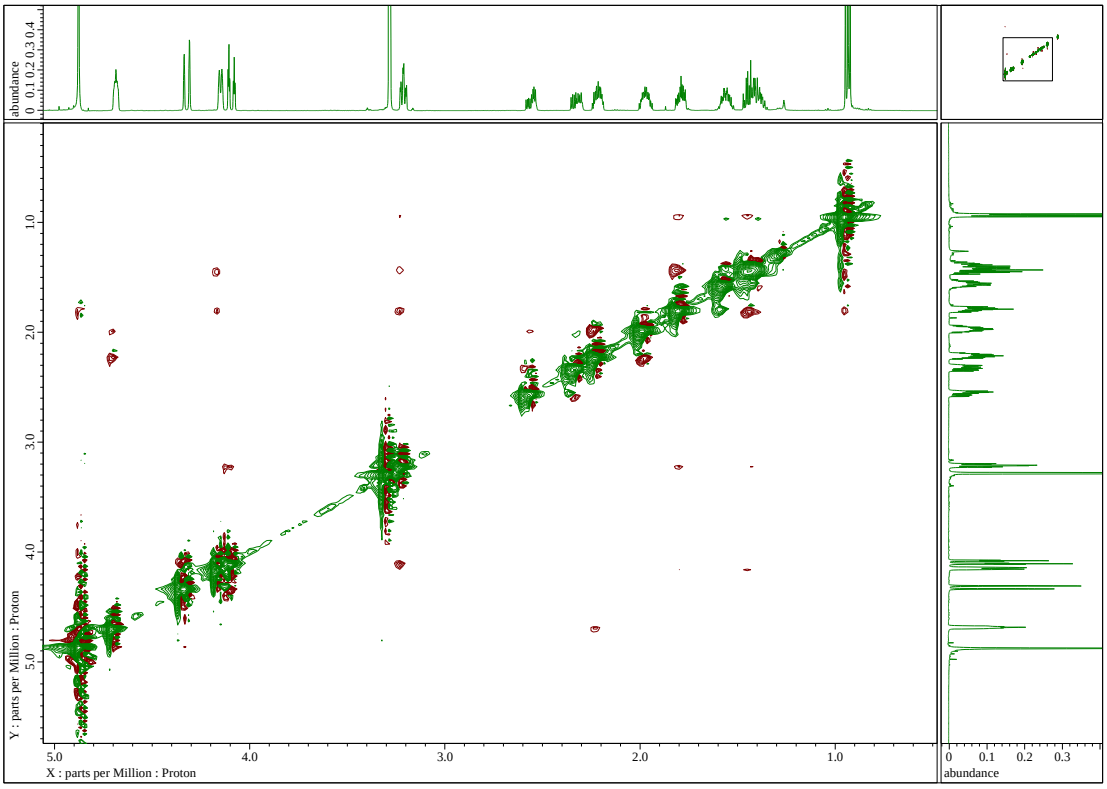

Figure S22: NOESY (CD_3OD) spectrum of phomopsiketone H (**2**)

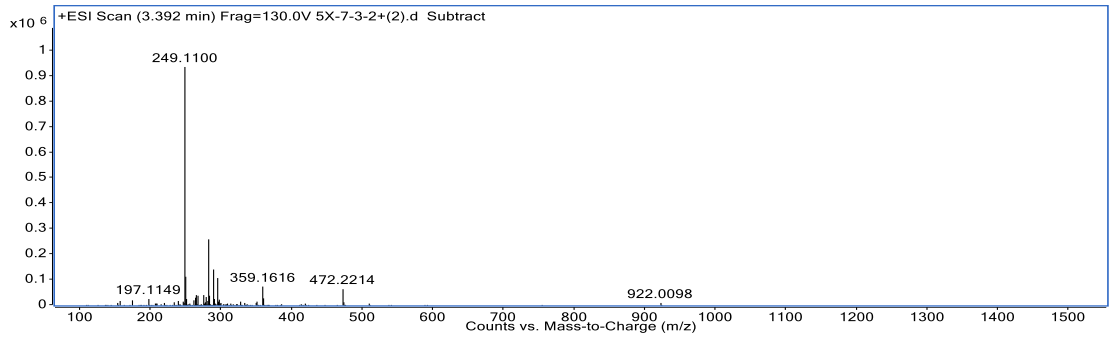

Figure S23: HRESIMS spectrum of phomopsiketone H (**2**)

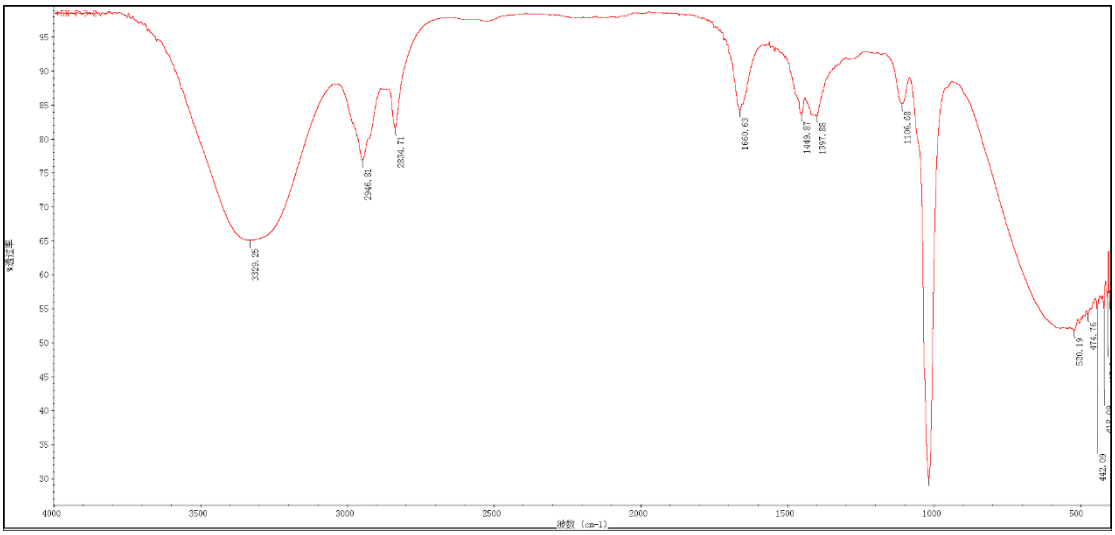

Figure S24: IR spectrum of phomopsiketone H (**2**)

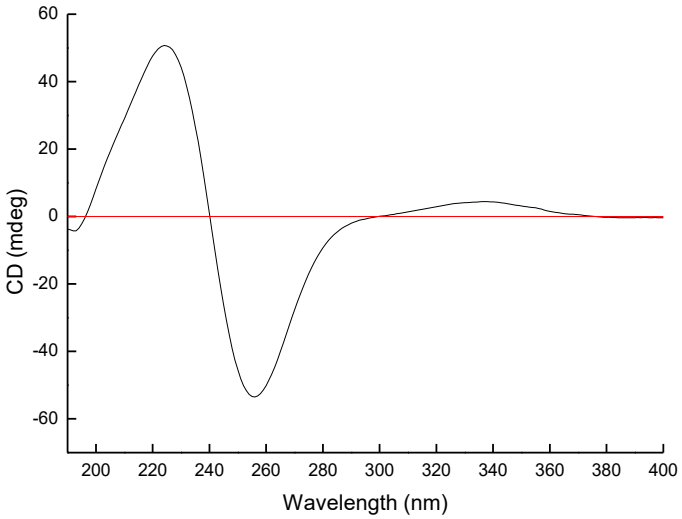

Figure S25: CD spectrum of phomopsiketone H (**2**)

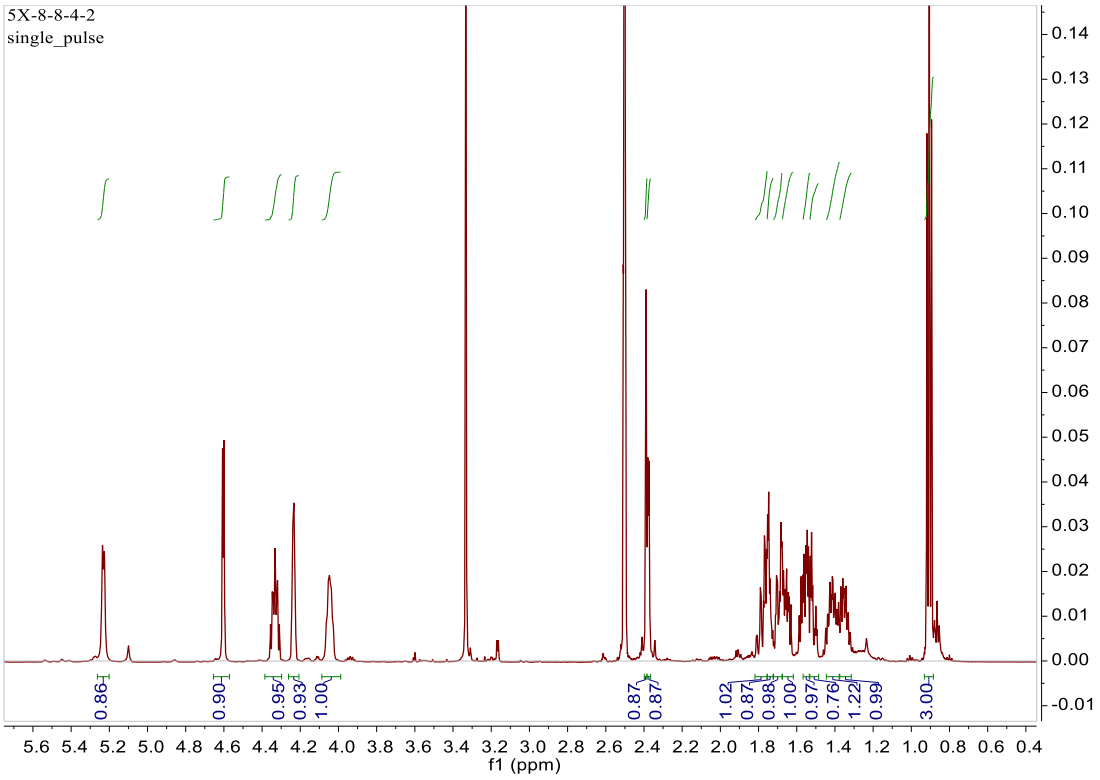

Figure S26: ^{1}H NMR (600 MHz, DMSO) spectrum of phomopsiketone I (**3**)

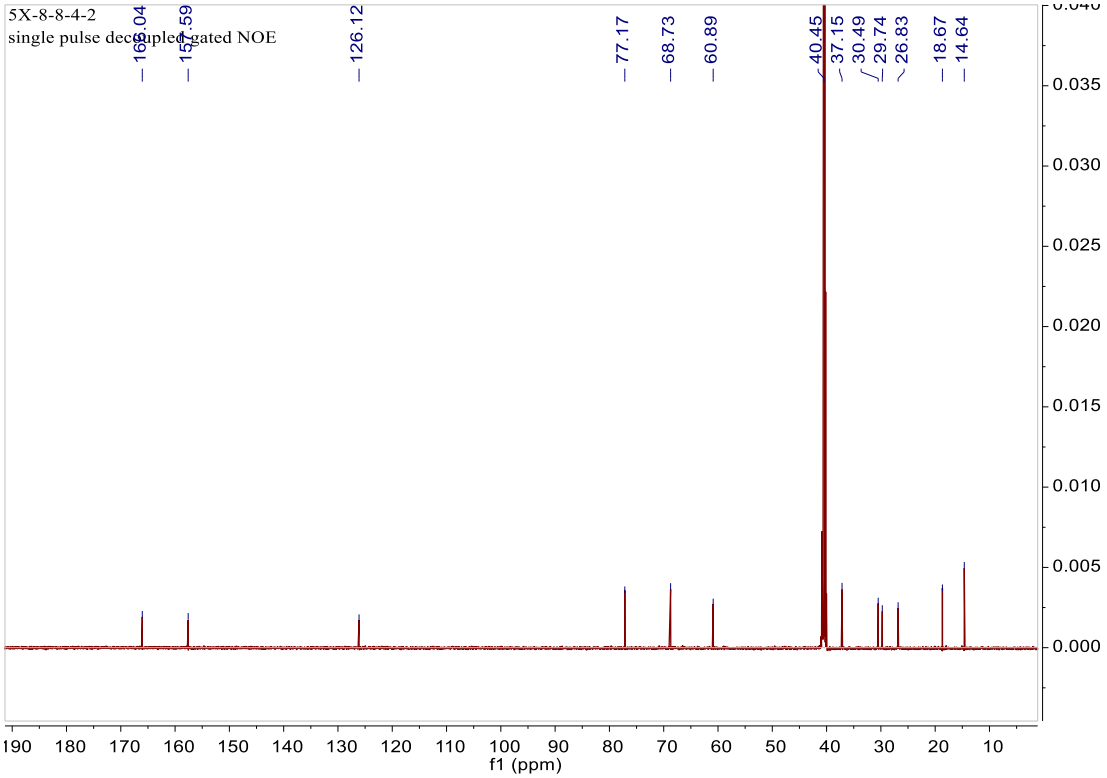

Figure S27: ^{13}C NMR (150 MHz, DMSO) spectrum of phomopsiketone I (**3**)

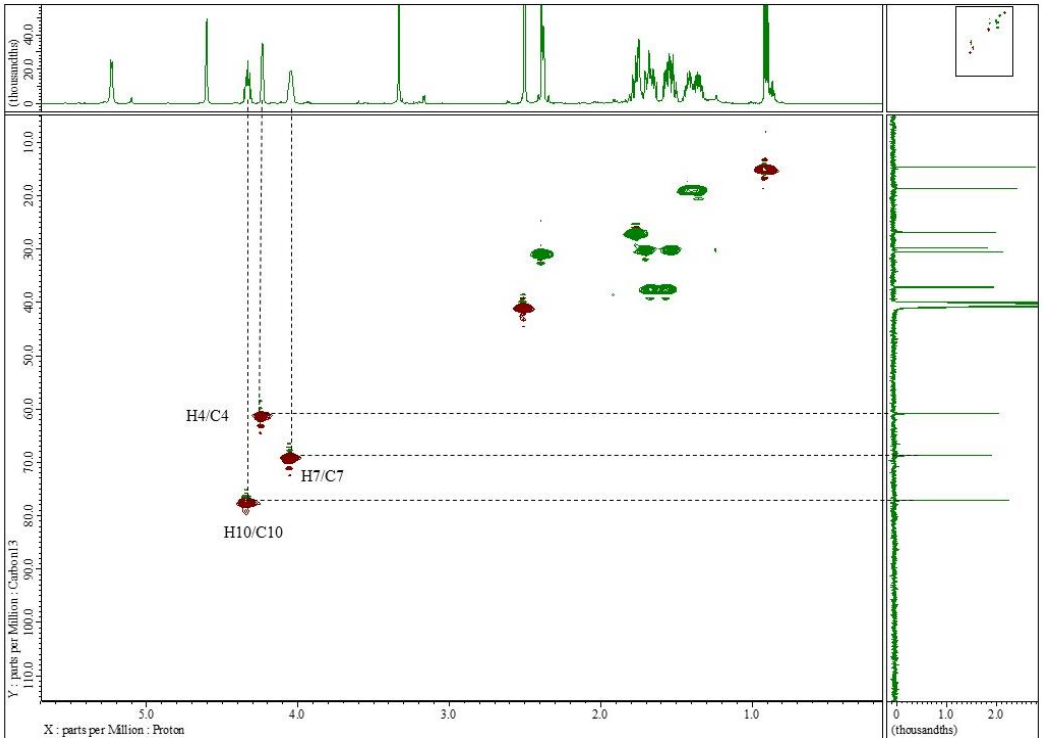

Figure S28: HSQC (DMSO) spectrum of phomopsiketone I (**3**)

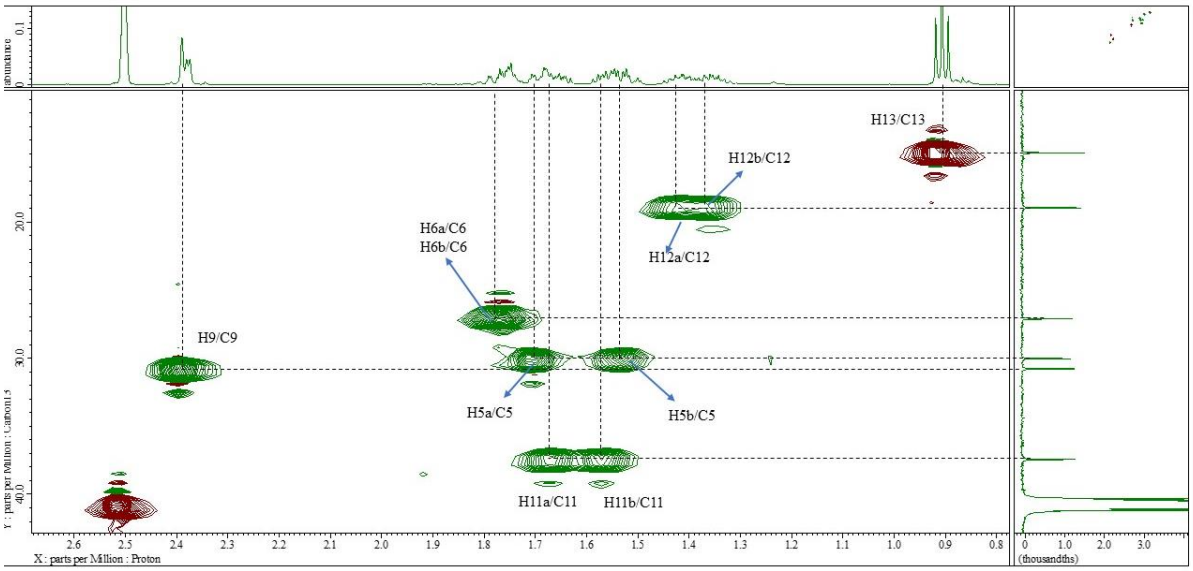

Figure S29: HSQC (DMSO) spectrum of phomopsiketone I (**3**) (From δ_C 5 ppm to δ_C 45 ppm)

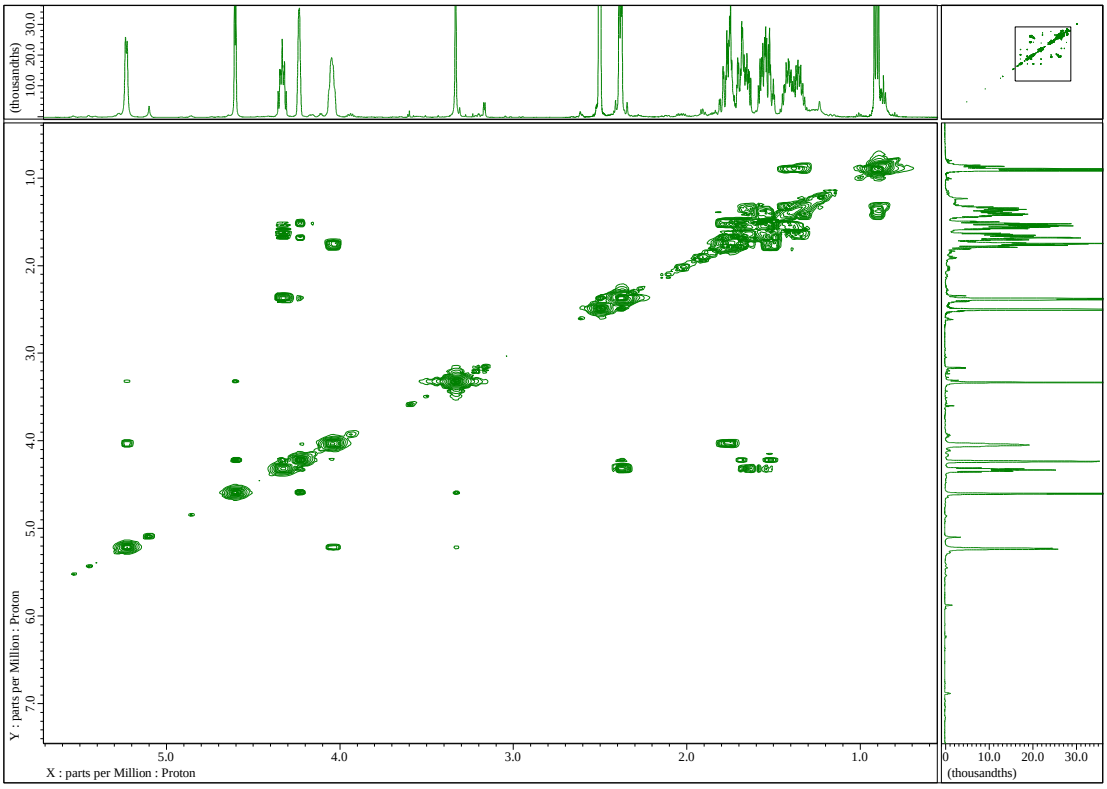

Figure S30: ^{1}H-^{1}H COSY (DMSO) spectrum of phomopsiketone I (**3**)

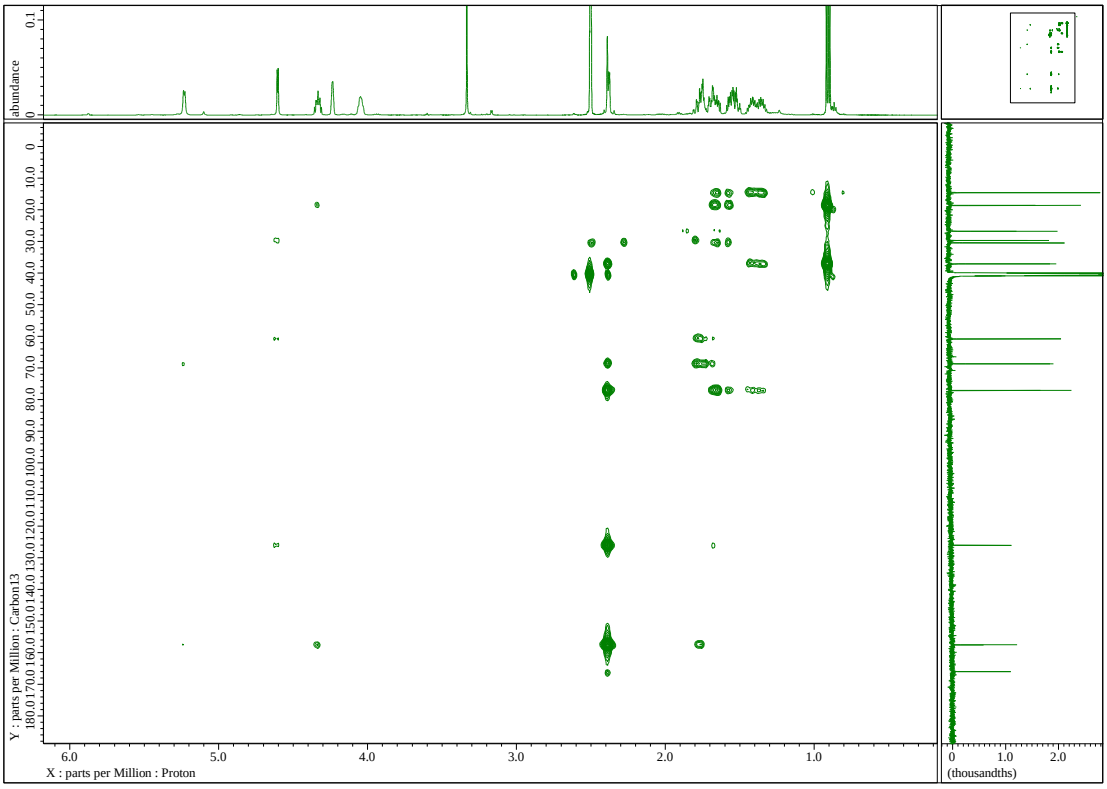

Figure S31: HMBC (DMSO) spectrum of phomopsiketone I (**3**)

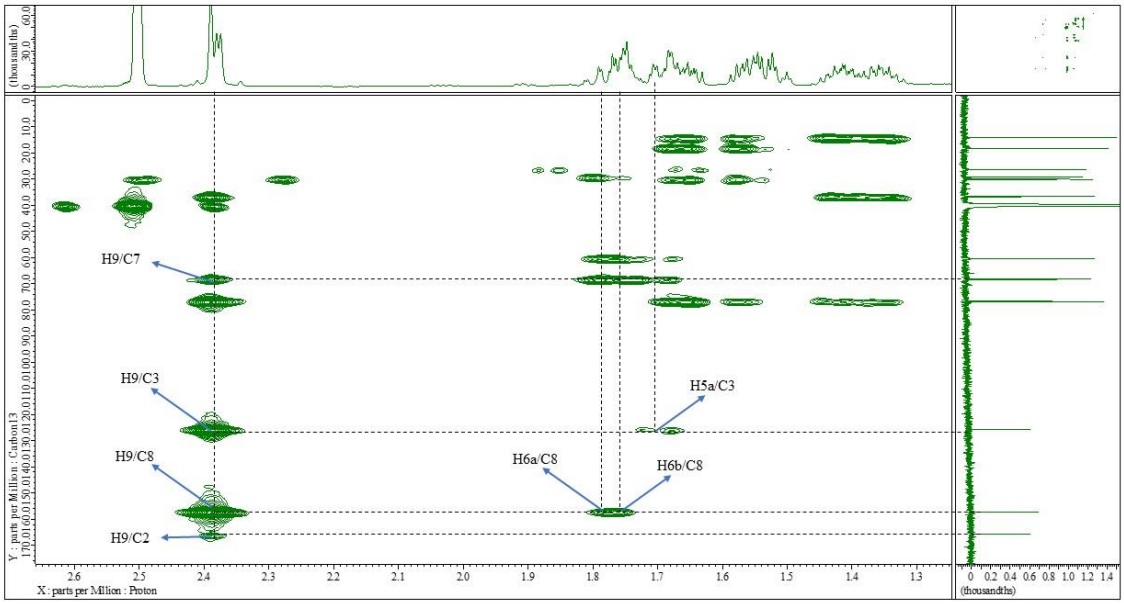

Figure S32: HMBC (DMSO) spectrum of phomopsiketone I (**3**) (From δ_H 1.3 ppm to δ_H 2.5 ppm)

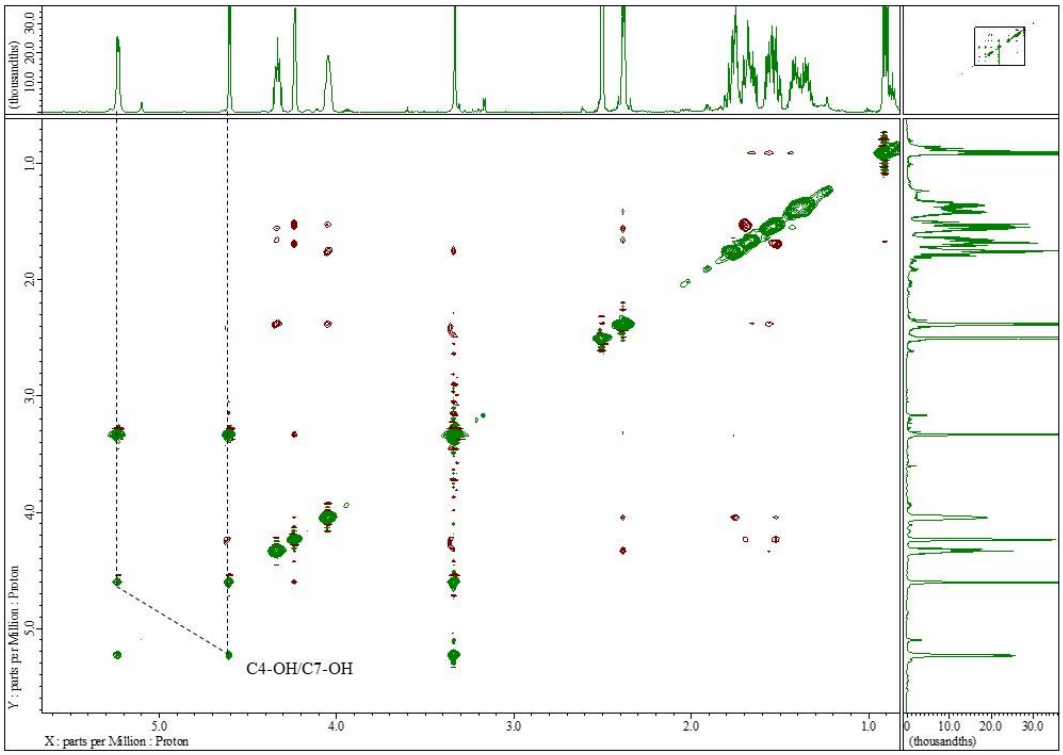

Figure S33: NOESY (DMSO) spectrum of phomopsiketone I (**3**)

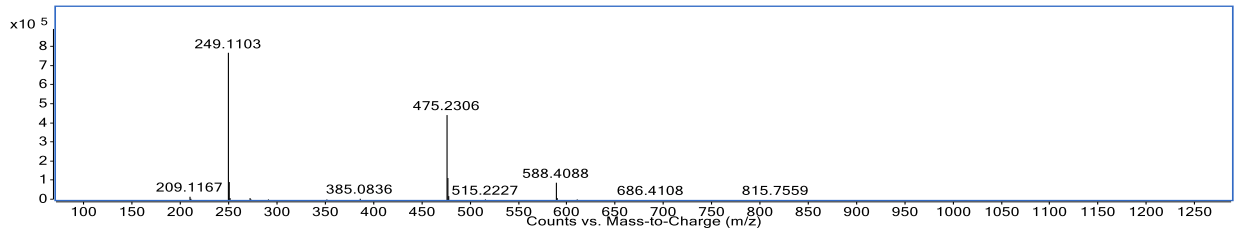

Figure S34: HRESIMS spectrum of phomopsiketone I (**3**)

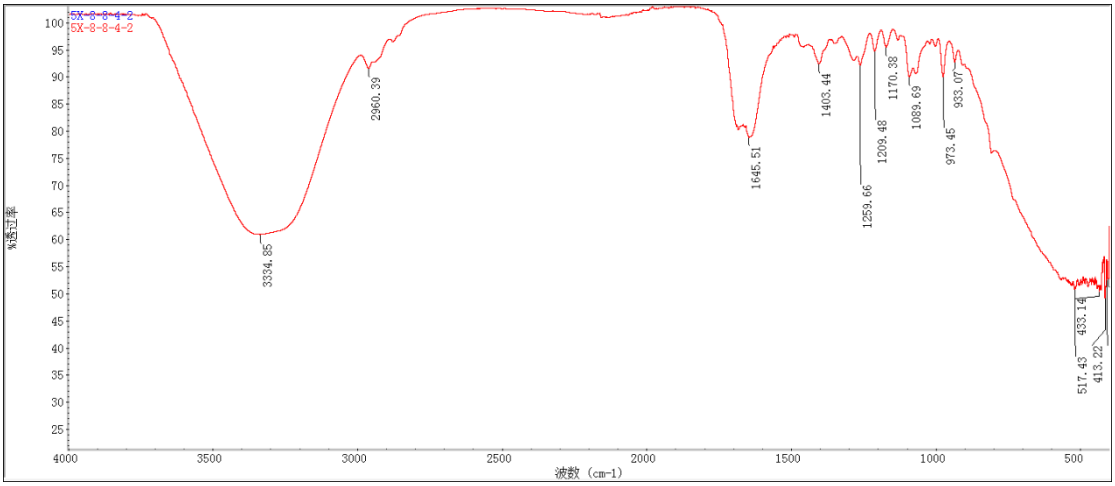

Figure S35: IR spectrum of phomopsiketone I (**3**)

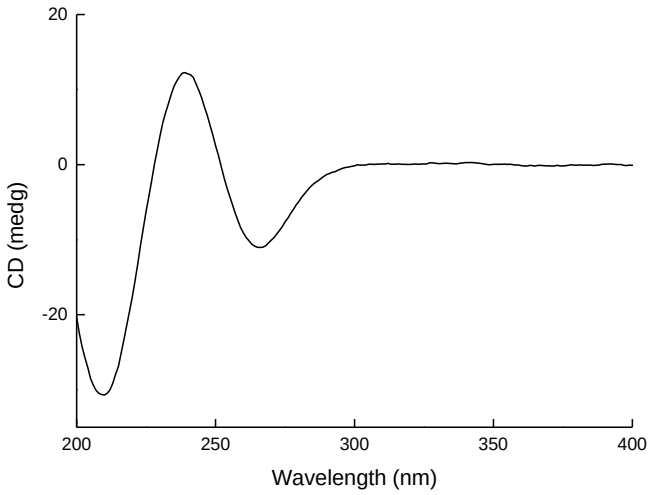

Figure S36: CD spectrum of phomopsiketone I (**3**)

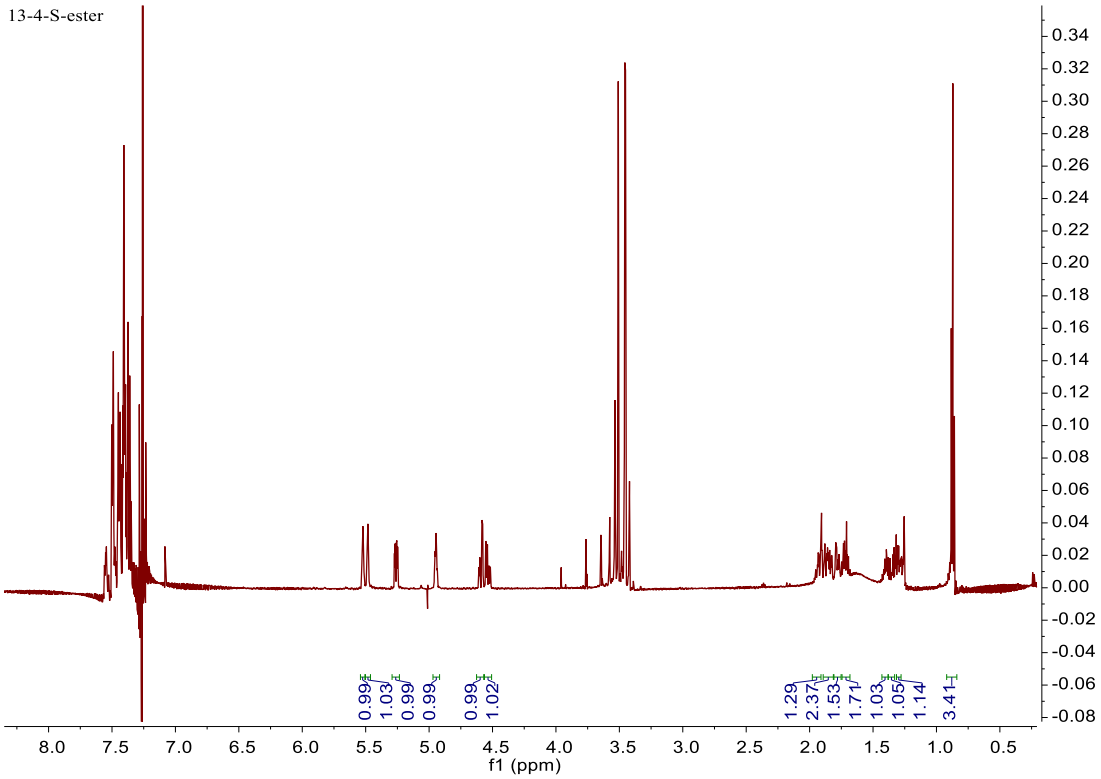

Figure S37: ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **1a**

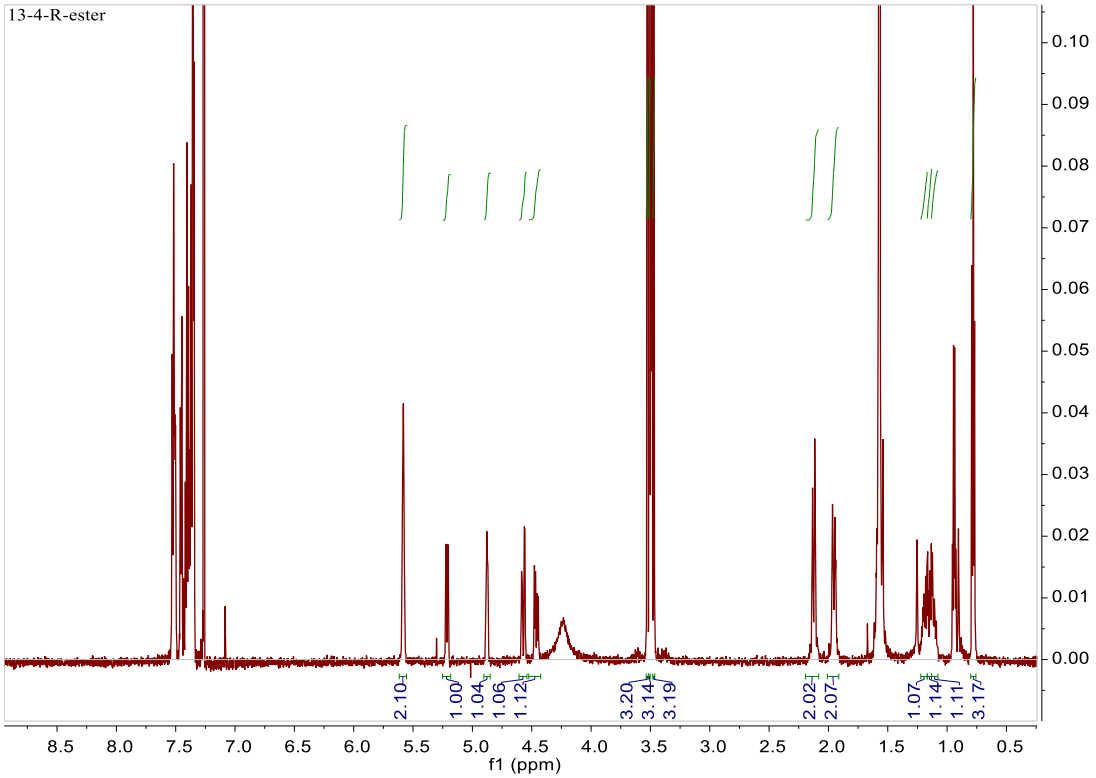

Figure S38: ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **1b**

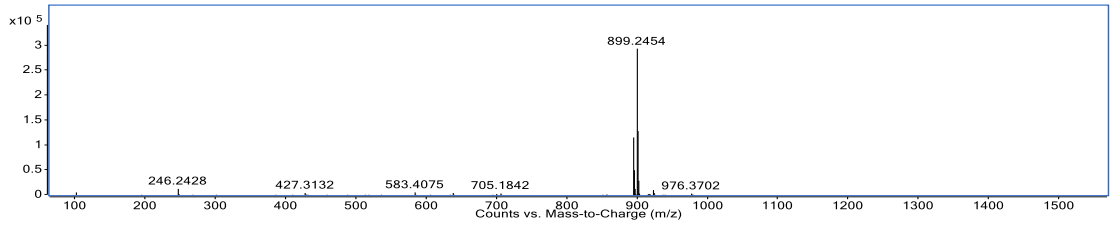

Figure S39: HRESIMS spectrum of **1a**

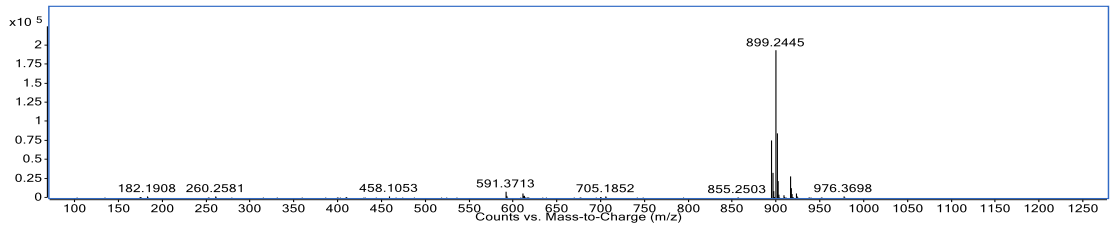

Figure S40: HRESIMS spectrum of **1b**

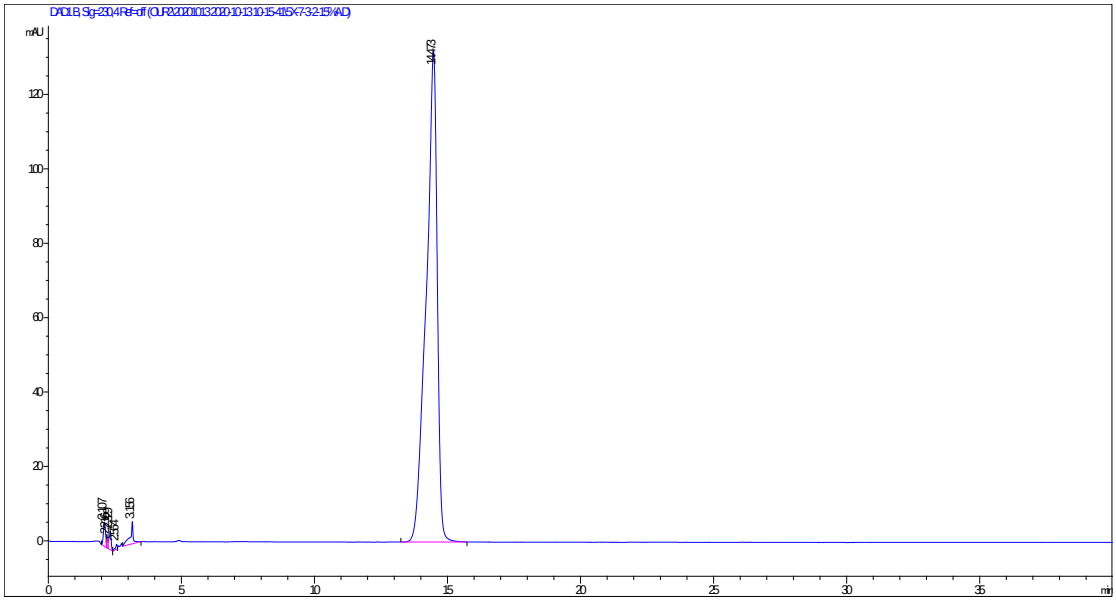

Figure S41: HPLC chromatogram of compound **2** (15%CH_3CN+H_20, 1 mL/min, 230 nm)

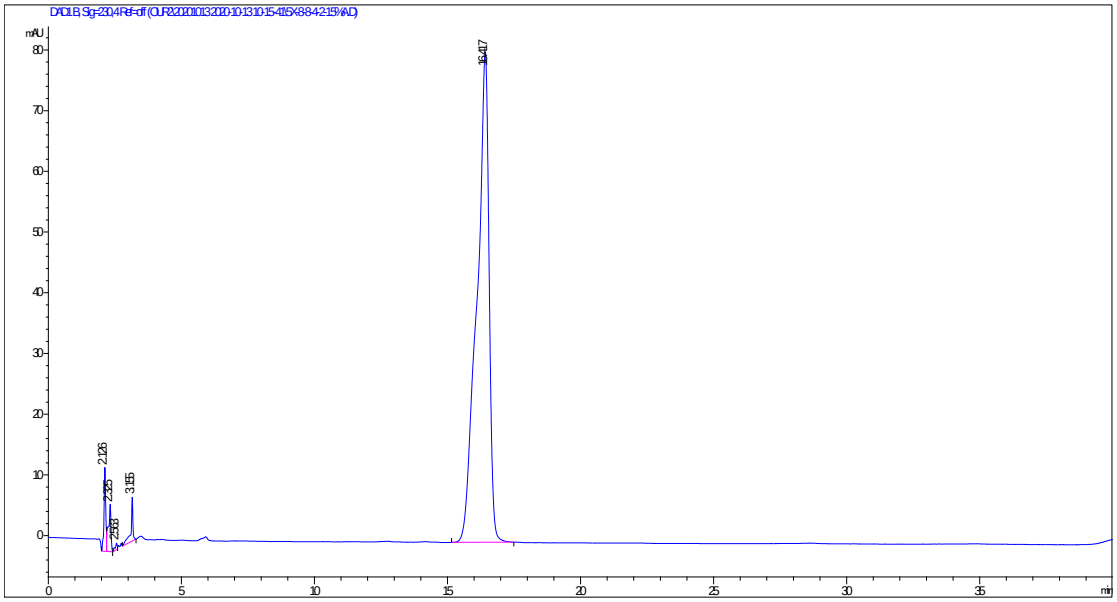

Figure S42: HPLC chromatogram of compound **3** (15%CH_3CN+H_20, 1 mL/min, 230 nm)

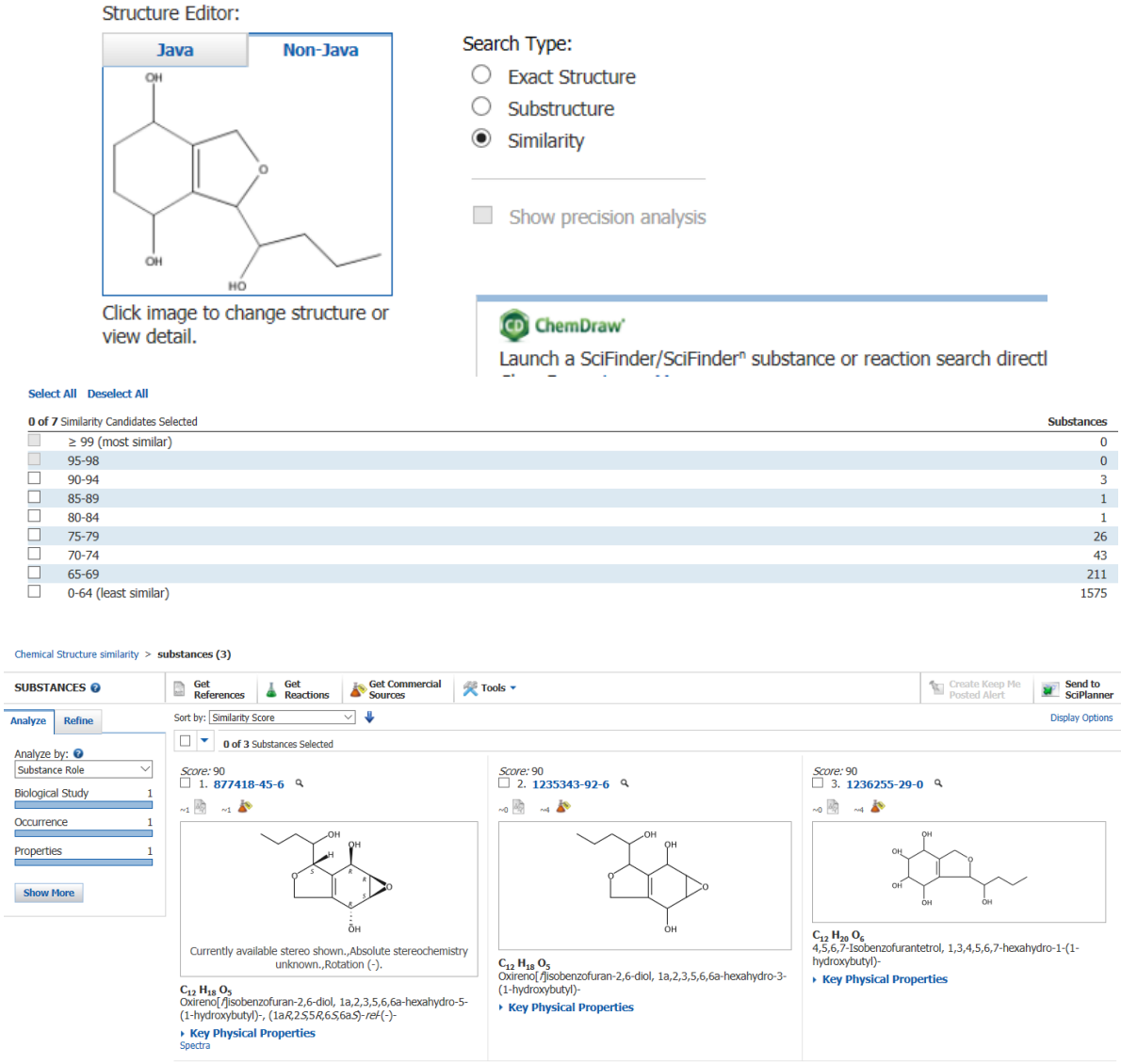

Figure S43: SciFinder Search Results of **1**

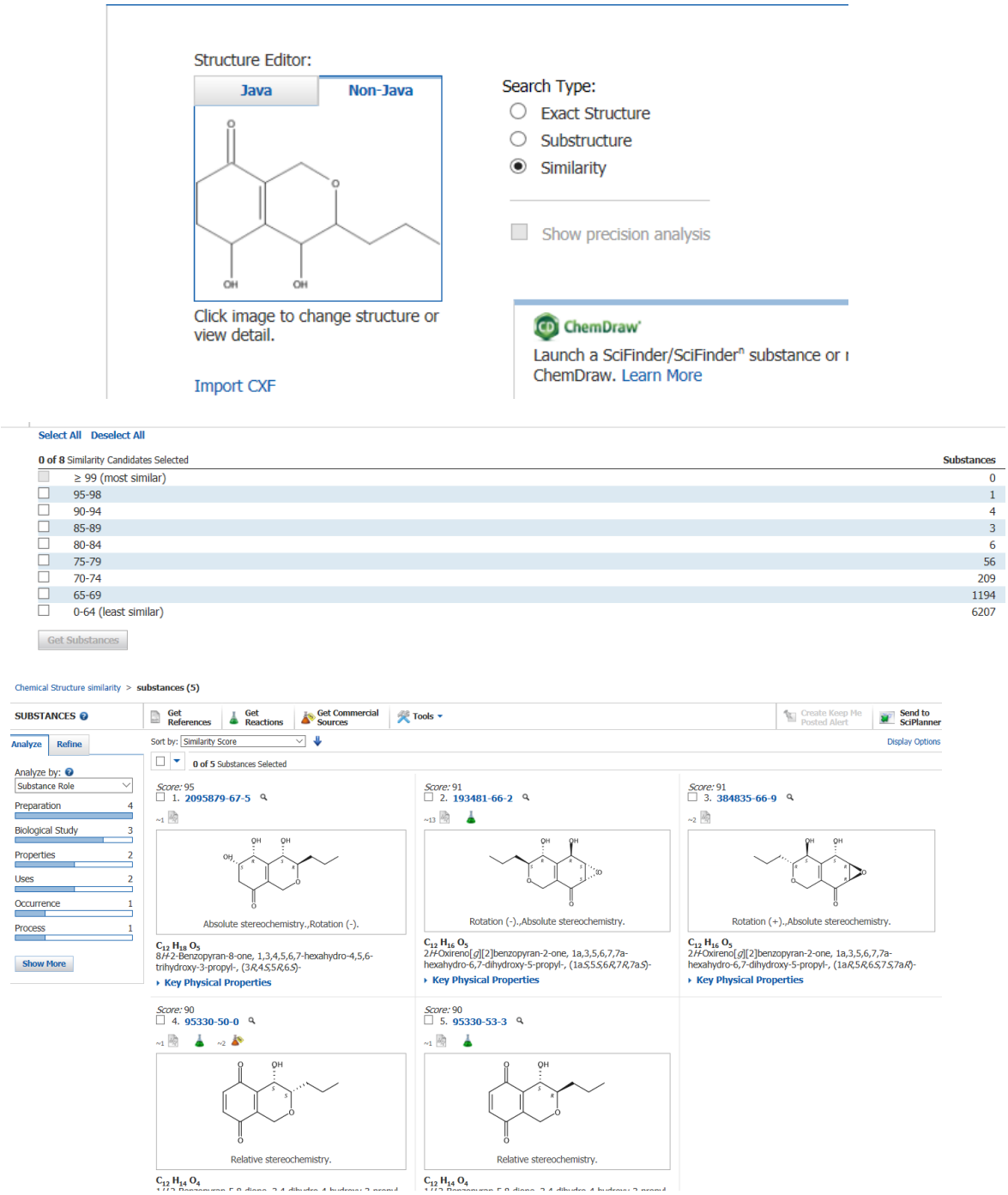

Figure S44: SciFinder Search Results of **2**

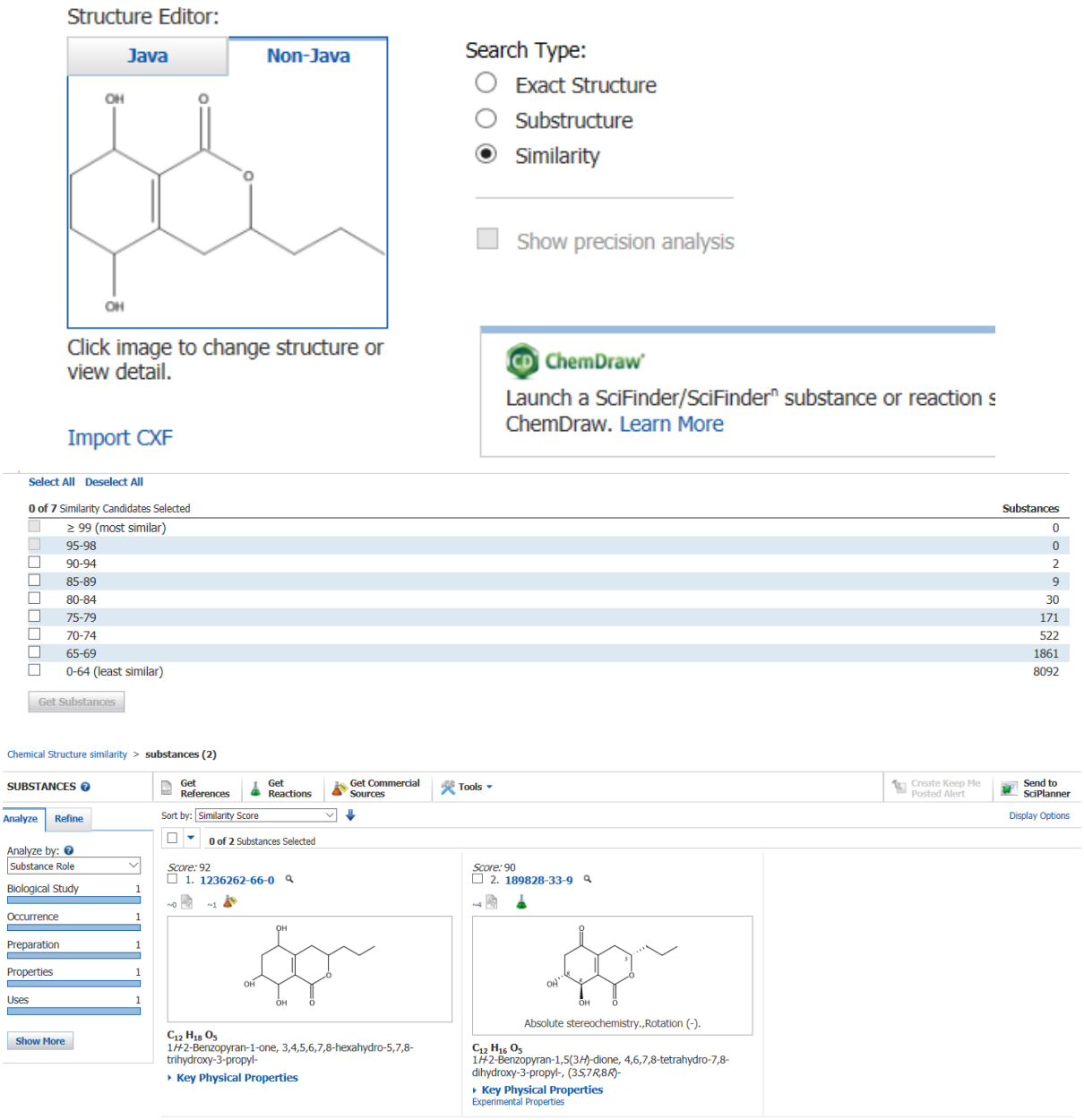

Figure S45: SciFinder Search Results of **3**