

Supporting Information

for

**Oscarellin, an Anthranilic Acid Derivative from a
Philippine Sponge *Oscarella stillans* as an Inhibitor
of Inflammatory Cytokines in Macrophages**

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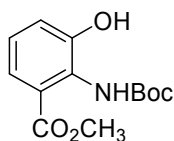
Figure S16. Effect of compound **1** on the cell proliferation.

1. General Methods

Commercially available reagents were used without additional purification, unless otherwise stated. All anhydrous solvents were distilled over CaH_2 or P_2O_5 or Na/benzophenone prior to reaction. All reactions were performed under an inert atmosphere of nitrogen or argon. Melting point was measured on a Electrothermal 9300 (Electrothermal Engineering LTD). ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 and 300 MHz spectrometer for CDCl_3 and CD_3OD solutions and chemical shifts are reported as parts per million (ppm) relative to residual CHCl_3 δ_{H} (7.26 ppm), CDCl_3 δ_{C} (77.0 ppm), CH_3OH δ_{H} (3.31 ppm), and CD_3OD δ_{C} (49.2 ppm), respectively, as internal standards. Resonance patterns are reported with the notations s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), and m (multiplet). In addition, the notation br is used to indicate a broad signal. Coupling constants (J) are reported in hertz (Hz). Thin layer chromatography was carried out using plates coated with Kieselgel 60F₂₅₄ (Merck). For flash column chromatography, Kieselgel 60 (Merck, 230-400 mesh) was used.

2. Experimental Procedure for Synthesis of 1 and Characterization Data for Intermediates and Product (1–3).

2.1. Methyl 2-(*tert*-butoxycarbonylamino)-3-hydroxybenzoate (2).



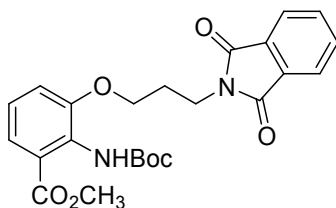
To a stirred solution of 3-hydroxyanthranilic acid (0.47 g, 3.069 mmol) in anhydrous MeOH (5.1 mL) was added SOCl_2 (0.11 mL, 1.228 mol) at 0 °C under N_2 . The reaction mixture was refluxed for 16 h. The resulting mixture was cooled in an ice bath, neutralized by the addition of solid NaHCO_3 (33 mg, 0.392 mmol) and concentrated in vacuo. The residual viscous oil was used without purification in the next step.

To a stirred solution of methyl 2-amino-3-hydroxybenzoate (about 0.52 g) from the previous step in 3 N NaOH (15 mL) was added di-*tert*-butyl dicarbonate (0.89 mL, 3.888

mmol) at 0 °C. The reaction mixture was stirred for 3 h at 60 °C. The resulting mixture was cooled to room temperature, neutralized by the careful addition of 6 N HCl (6 mL). The aqueous layer was extracted with CH₂Cl₂ (30 mL×3) and the organic layer was washed with brine, dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (hexane-EtOAc, 4:1) to afford 0.58 g (71%) of **2** as a pale yellow solid.

R_f = 0.30 (hexane-EtOAc, 4:1); ¹H NMR (CDCl₃, 300 MHz) δ_H 1.60 (27H, s), 3.92 (3H, s), 5.91 (2H, br s), 6.57 (1H, t, J = 8.1 Hz), 7.28 (1H, dd, J = 7.8, 1.5 Hz), 7.79 (1H, dd, J = 8.1, 1.5 Hz); ¹³C NMR (CDCl₃, 125 MHz) δ_C 27.89, 51.91, 84.28, 112.63, 115.15, 126.51, 128.72, 138.67, 143.20, 151.54, 168.39.

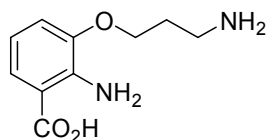
2.2. Methyl 2-(*tert*-butoxycarbonylamino)-3-[3-(1,3-dioxoisindolin-2-yl)propoxy]benzoate (**3**).



To a stirred solution of **2** (0.29 g, 1.085 mmol) in anhydrous acetone (5.4 mL) was added K₂CO₃ (0.5 g, 3.255 mmol) and *N*-(3-bromopropyl)phthalimide (0.38 g, 1.411 mmol) at room temperature under N₂. The reaction mixture was refluxed for 24 h then cooled in an ice bath. The resulting mixture was filtered through a Celite pad, and then concentrated in vacuo. The residue was purified by column chromatography (hexane-EtOAc, 2:1) to afford 0.46 g (93%) of **3** as yellow oil.

R_f = 0.28 (hexane-EtOAc, 2:1); ¹H NMR (CDCl₃, 300 MHz) δ_H 1.55 (27H, s), 2.26 (2H, m), 3.91 (3H, s), 3.95 (2H, t, J = 6.6 Hz), 4.13 (2H, t, J = 6.3 Hz), 7.06 (1H, dd, J = 8.1, 1.5 Hz), 7.13 (1H, t, J = 8.1 Hz), 7.39 (1H, br), 7.44 (1H, dd, J = 7.8, 1.5 Hz), 7.76 (1H, m), 7.88 (1H, m); ¹³C NMR (CDCl₃, 125 MHz) δ_C 28.46, 28.70, 35.30, 52.36, 66.74, 80.59, 115.82, 122.63, 123.53, 124.59, 125.81, 127.54, 132.35, 134.20, 151.54, 153.45, 168.05, 168.53.

2.3. 2-Amino-3-(3'-aminopropoxy)benzoic acid (**1**).



To a stirred solution of **2** (0.32 g, 0.704 mmol) in 1,4-dioxane (3.5 mL) was added conc. HCl (1.2 mL) at 0 °C under N₂. The reaction mixture was stirred for 48 h at 100 °C and then concentrated in vacuo. The residue was purified by column chromatography (CHCl₃-MeOH, 2:1) and recrystallization (acetone) to afford 0.13 g (75%) of **1** as its hydrochloride salt, a white solid.

R_f = 0.20 (CHCl₃-MeOH, 2:1); mp 206.9–207.4; ¹H NMR (CD₃OD, 300 MHz) δ_H 2.24 (2H, p, J = 7.0 Hz), 3.24 (2H, t, J = 7.4 Hz), 4.21 (2H, t, J = 6.0 Hz), 6.60 (1H, t, J = 8.0 Hz), 7.01 (1H, dd, J = 7.7, 1.4 Hz), 7.51 (1H, dd, J = 8.4, 1.2 Hz); ¹³C NMR (CD₃OD, 125 MHz) δ_C 28.44, 38.66, 66.82, 112.46, 115.68, 115.80, 124.79, 142.15, 147.56, 171.32.

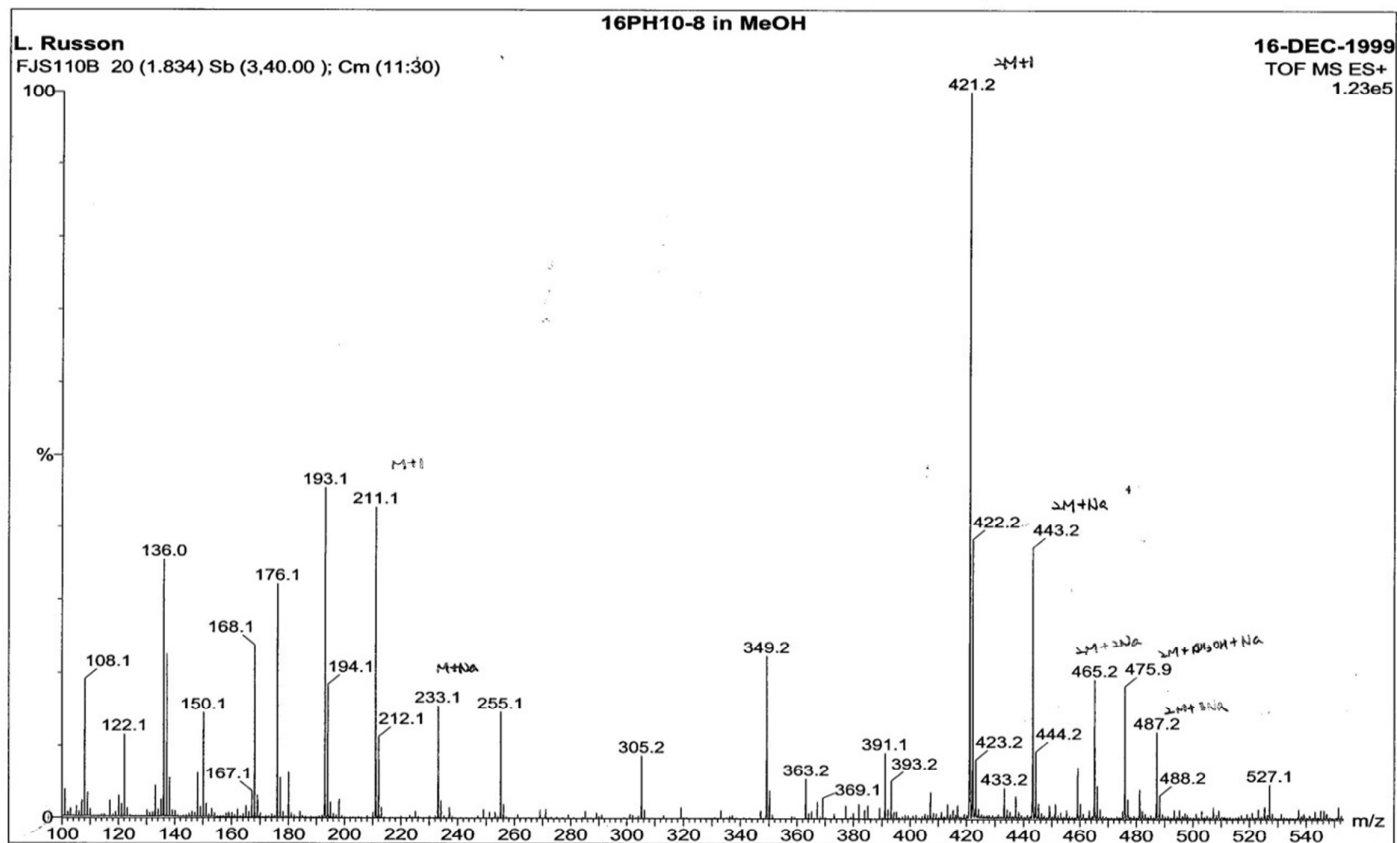


Figure S1. ESIMS spectrum of compound 1.

[Mass Spectrum]
Data : 20160415-KIS-712-005 Date : 15-Apr-2016 15:29
Sample :
Note : Jeol-JMS-700
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion (MF-Linear)
RT : 0.34 min Scan# : 11
BP : m/z 185.0722 Int. : 331.26
Output m/z range : 50.0000 to 270.8227 Cut Level : 0.00 %
4140200

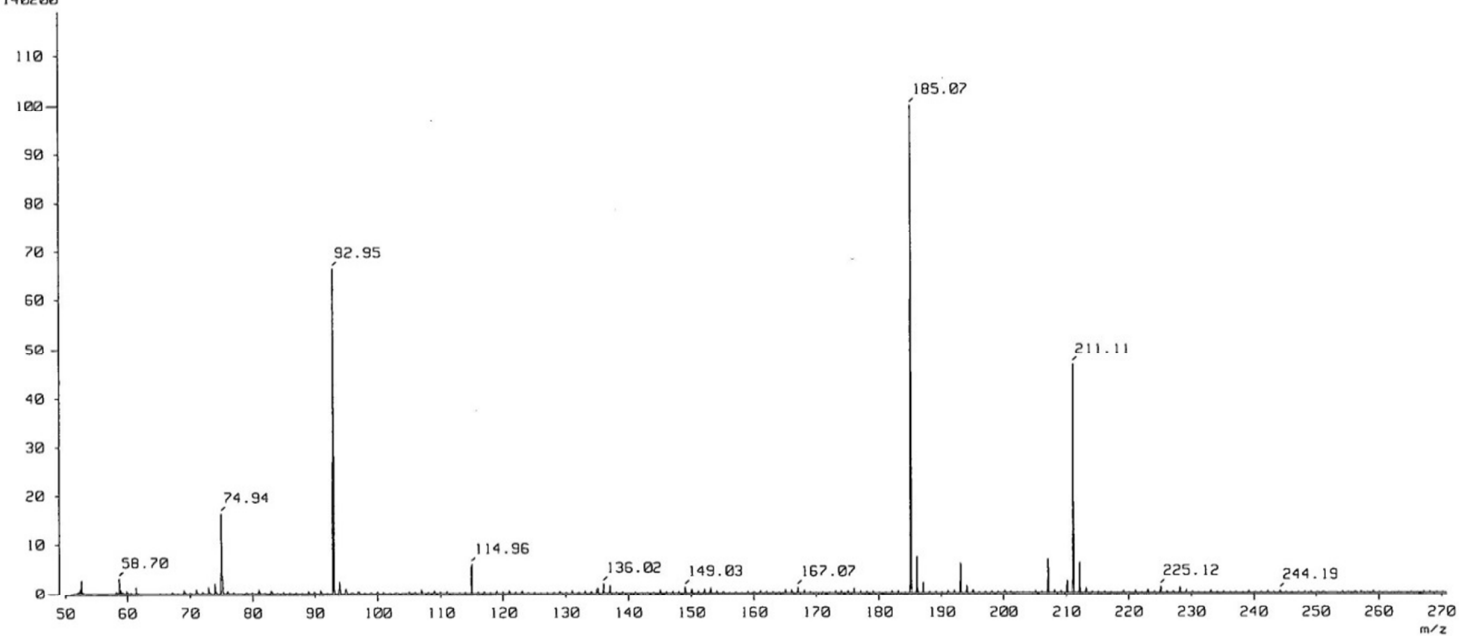


Figure S2. FABMS spectrum of compound **1**.

[Mass Spectrum]
Data : 20160414-KIS-712-001 Date : 14-Apr-2016 16:06
Sample: -
Note : -
Inlet : Reserv. Ion Mode : EI+
Spectrum Type : Normal Ion [MF-Linear]
RT : 6.00 min Scan# : 181
BP : m/z 227.9270 Int. : 399.97
Output m/z range : 210.0155 to 210.2467 Cut Level : 0.00 %

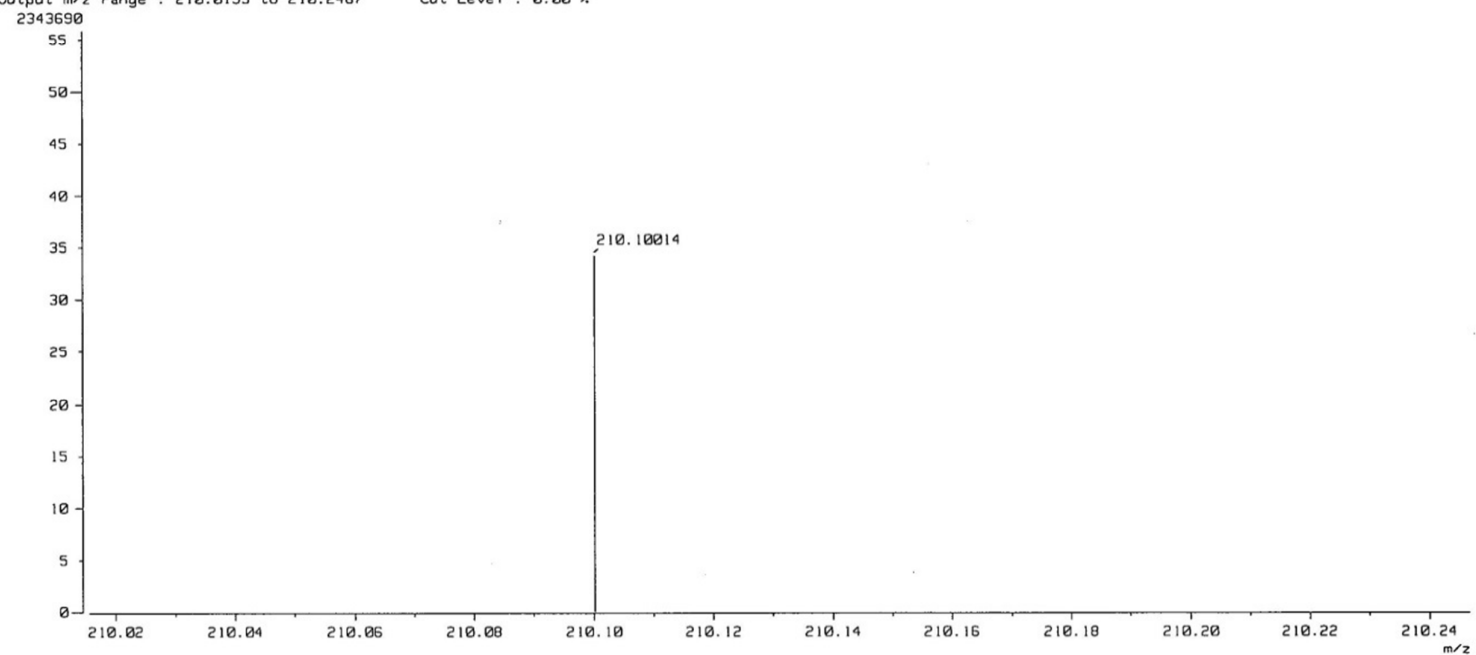


Figure S3. HREIMS spectrum of compound **1**.

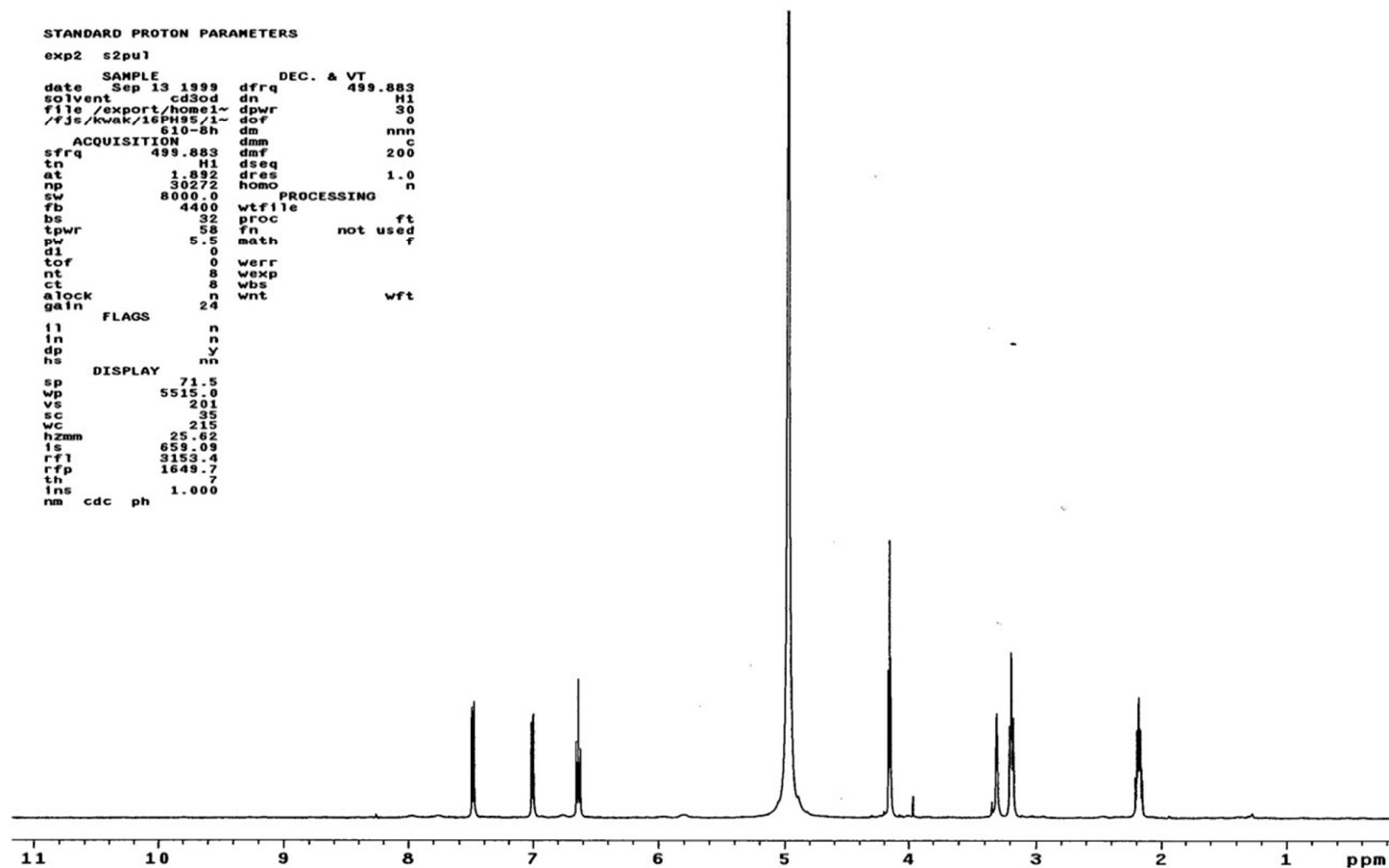


Figure S4. ^1H NMR spectrum of compound **1** (CD_3OD , 500 MHz).

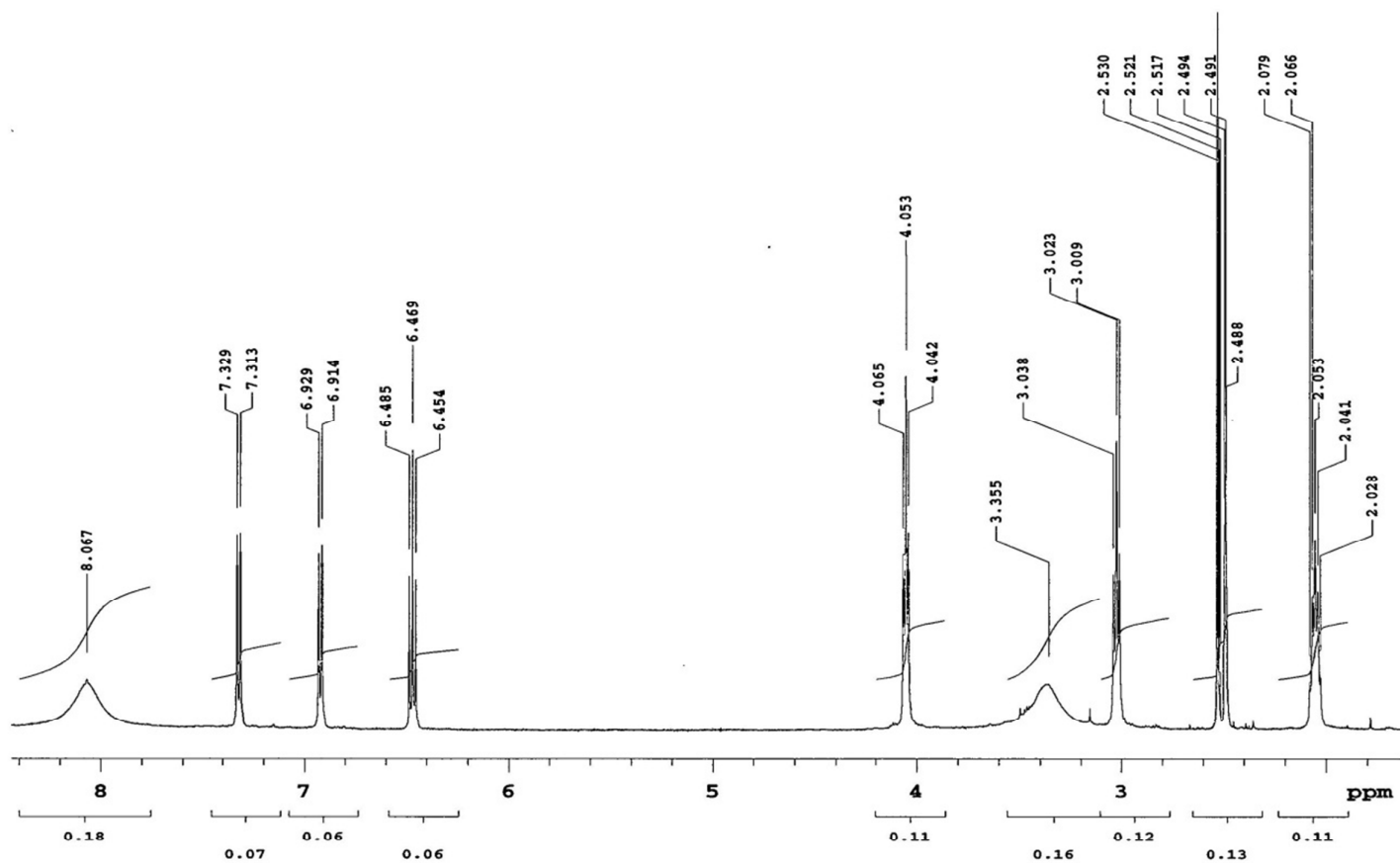


Figure S5. ^1H NMR spectrum of compound 1 ($\text{DMSO}-d_6$, 500 MHz).

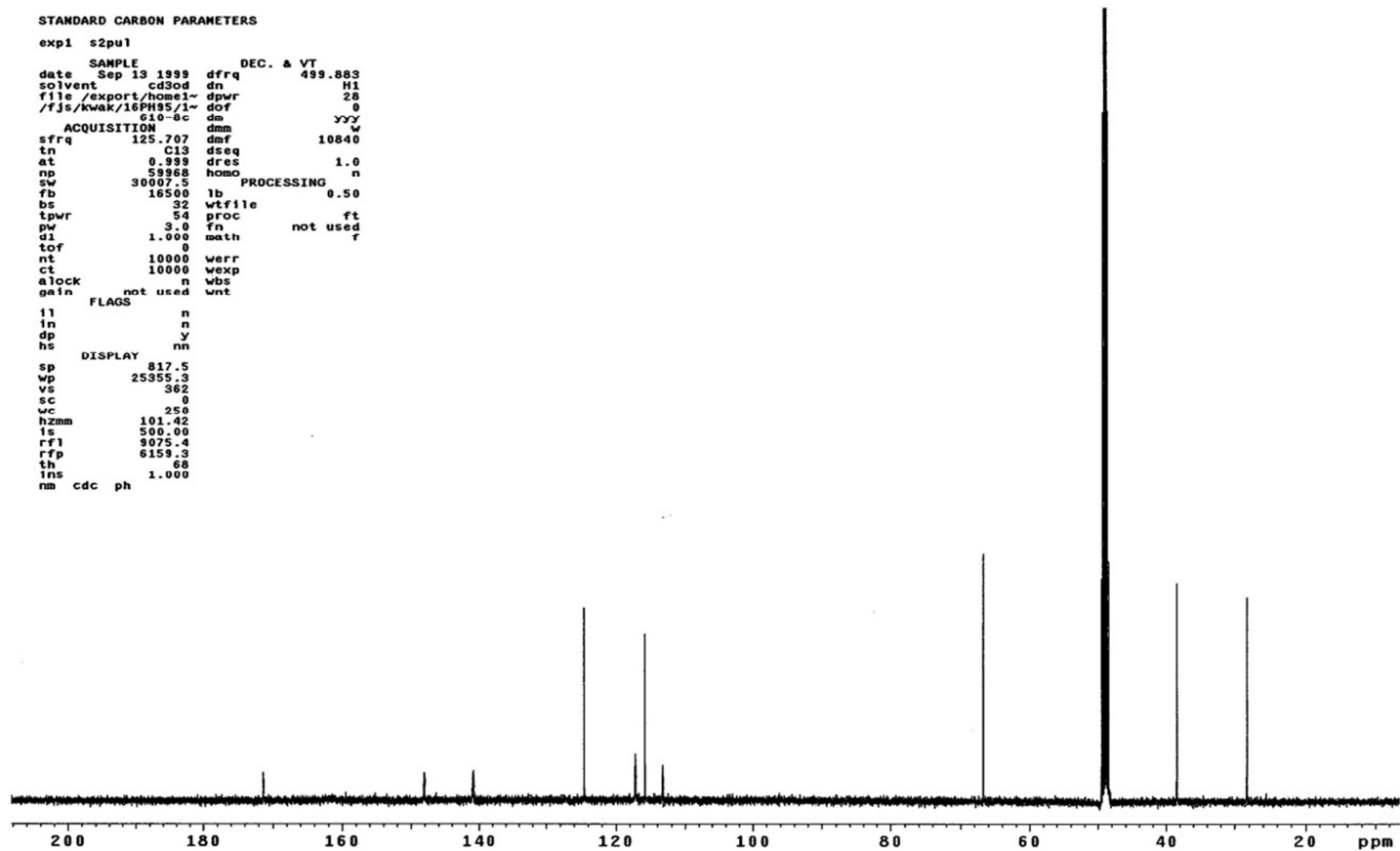


Figure S6. ^{13}C NMR spectrum of compound 1 (CD_3OD , 125 MHz).

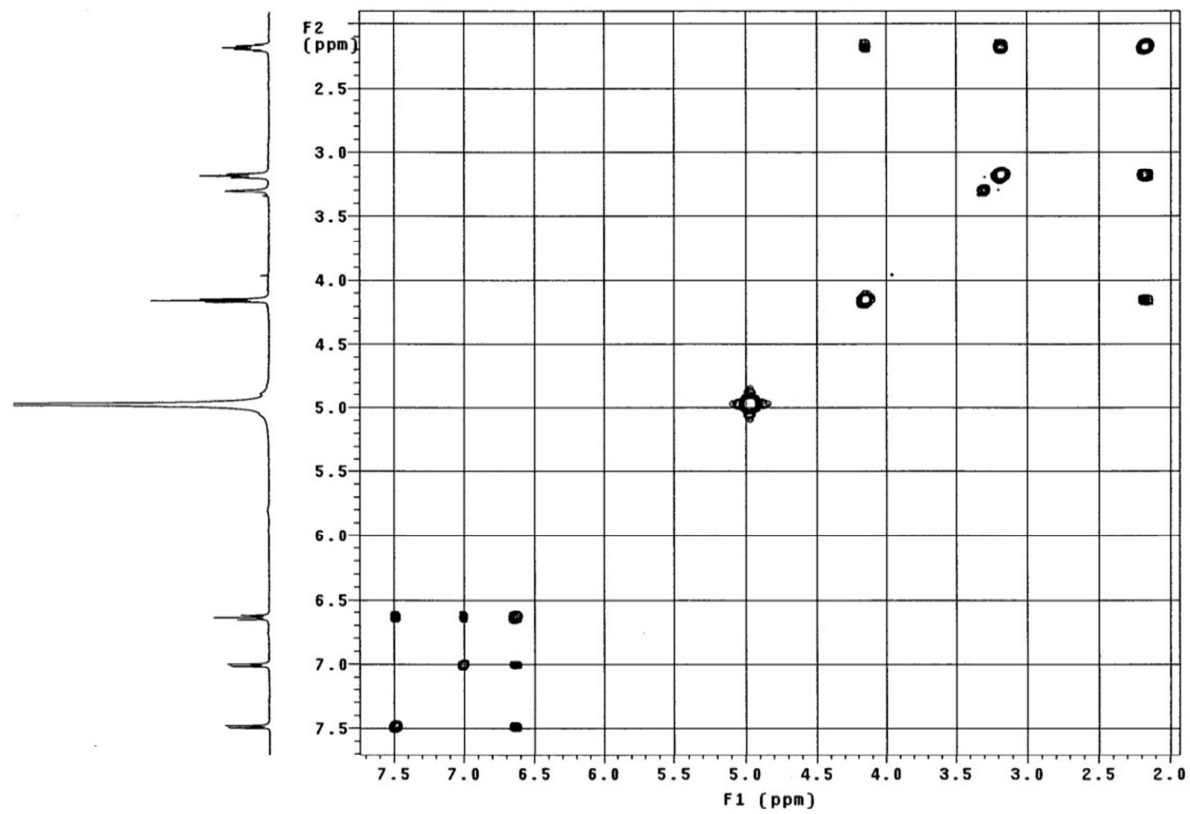


Figure S7. ^1H - ^1H COSY spectrum of compound **1** (CD_3OD , 500 MHz).

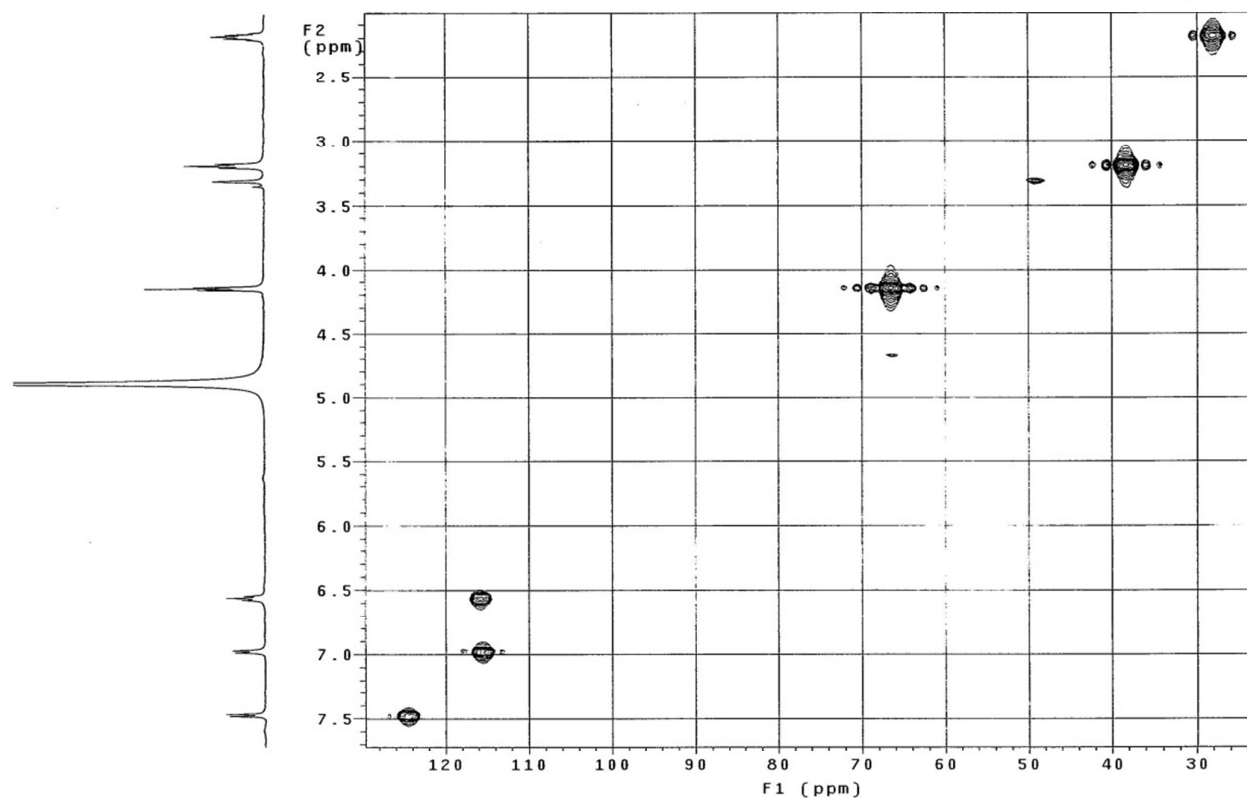


Figure S8. HMQC spectrum of compound **1** (CD_3OD , 500 MHz).

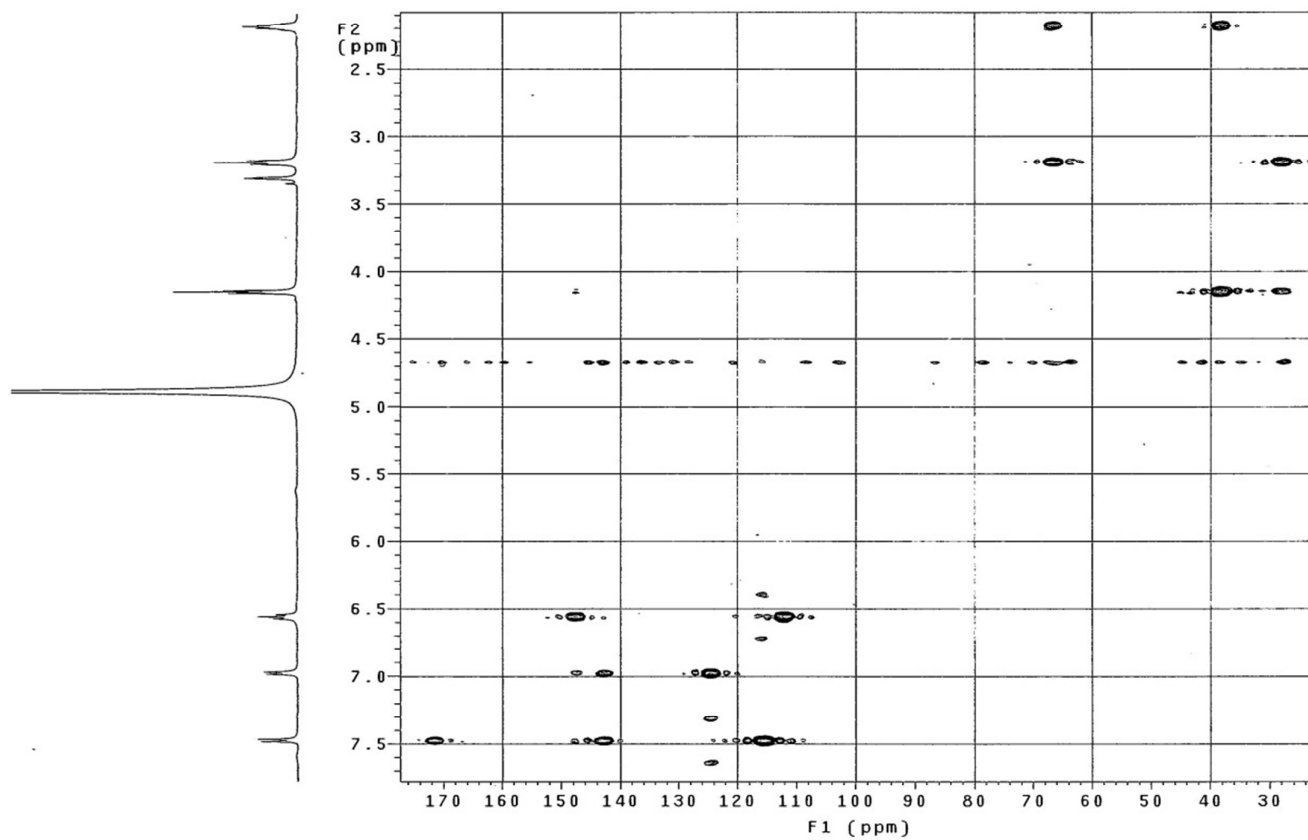


Figure S9. HMBC spectrum of compound **1** (CD₃OD, 500 MHz).

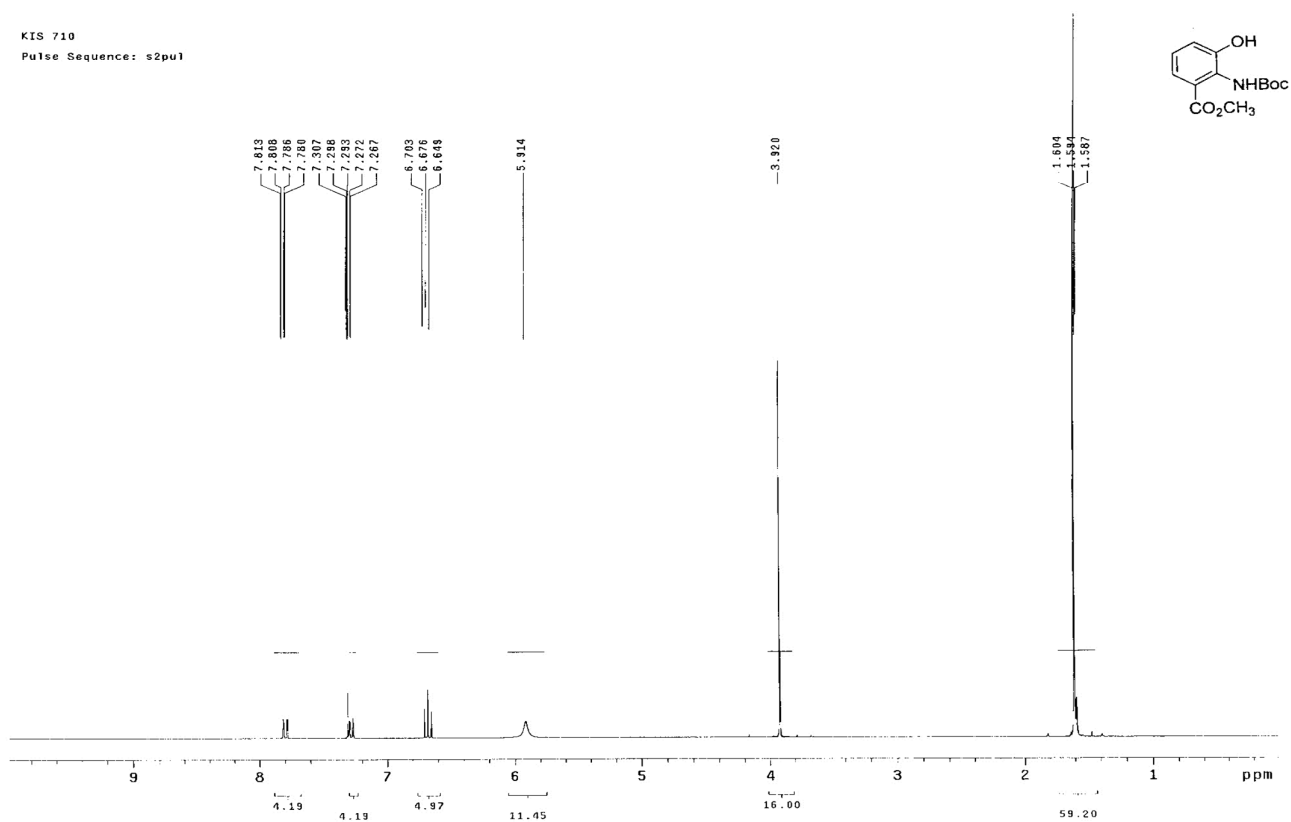


Figure S10. ^1H NMR spectrum of compound **2** (CDCl_3 , 300 MHz).

KIS-710-13C
Pulse Sequence: s2pu1

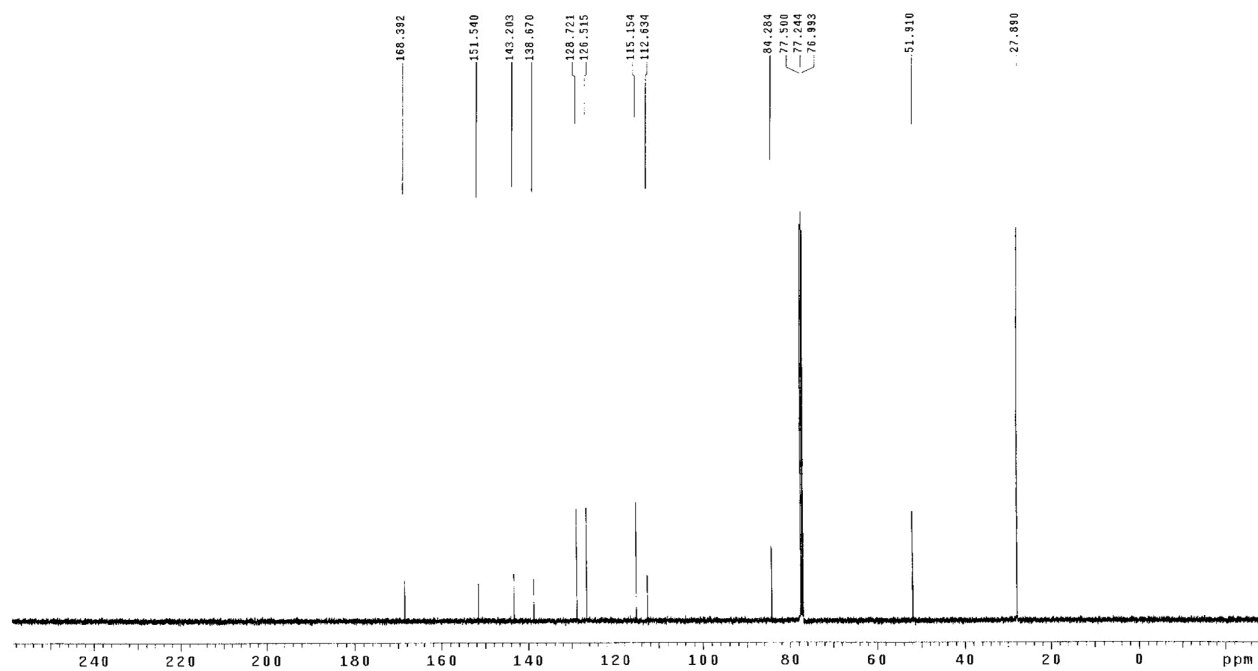
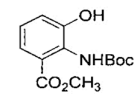


Figure S11. ^{13}C NMR spectrum of compound **2** (CDCl_3 , 125 MHz).

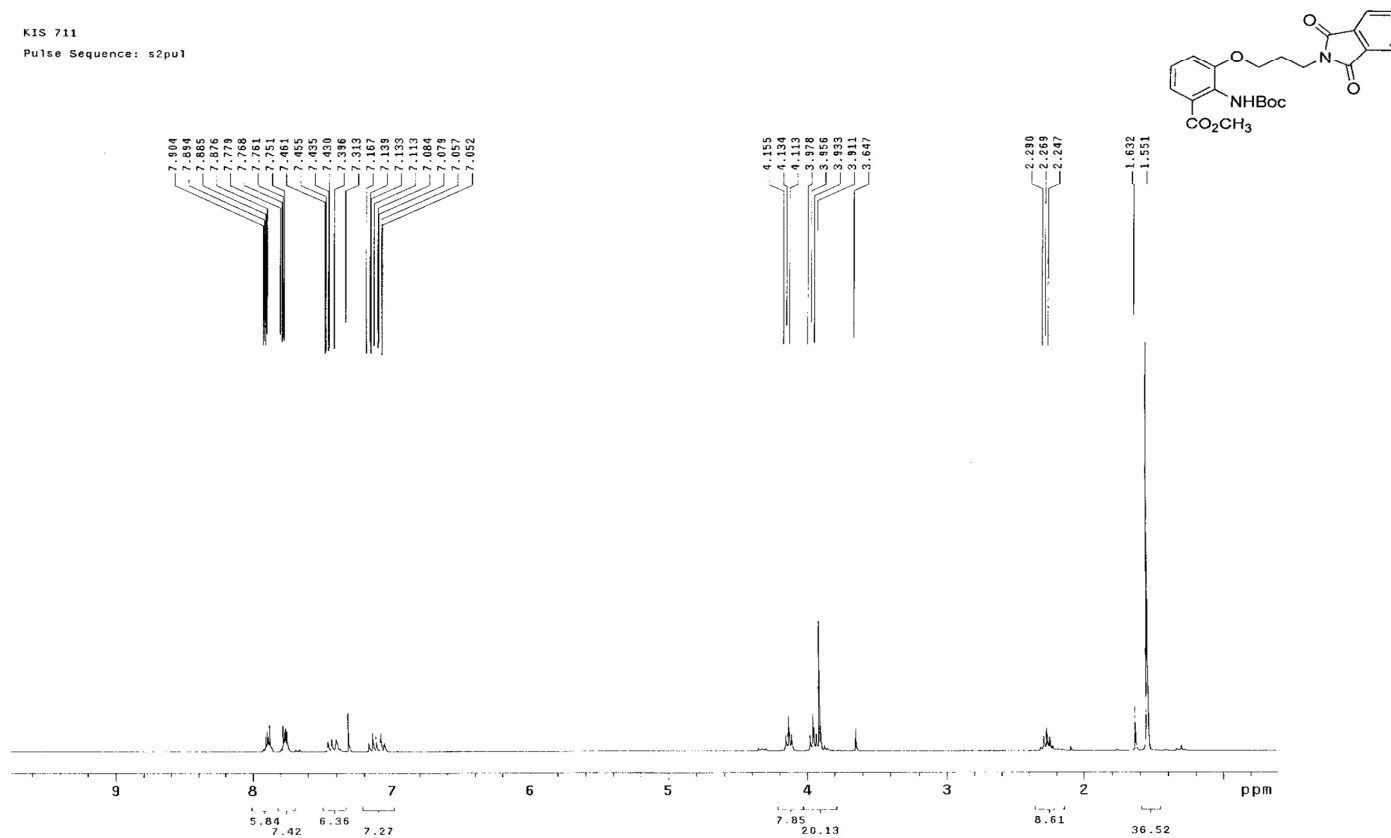


Figure S12. ¹H NMR spectrum of compound **3** (CDCl₃, 300 MHz).

KIS-711-13C
Pulse Sequence: s2pu1

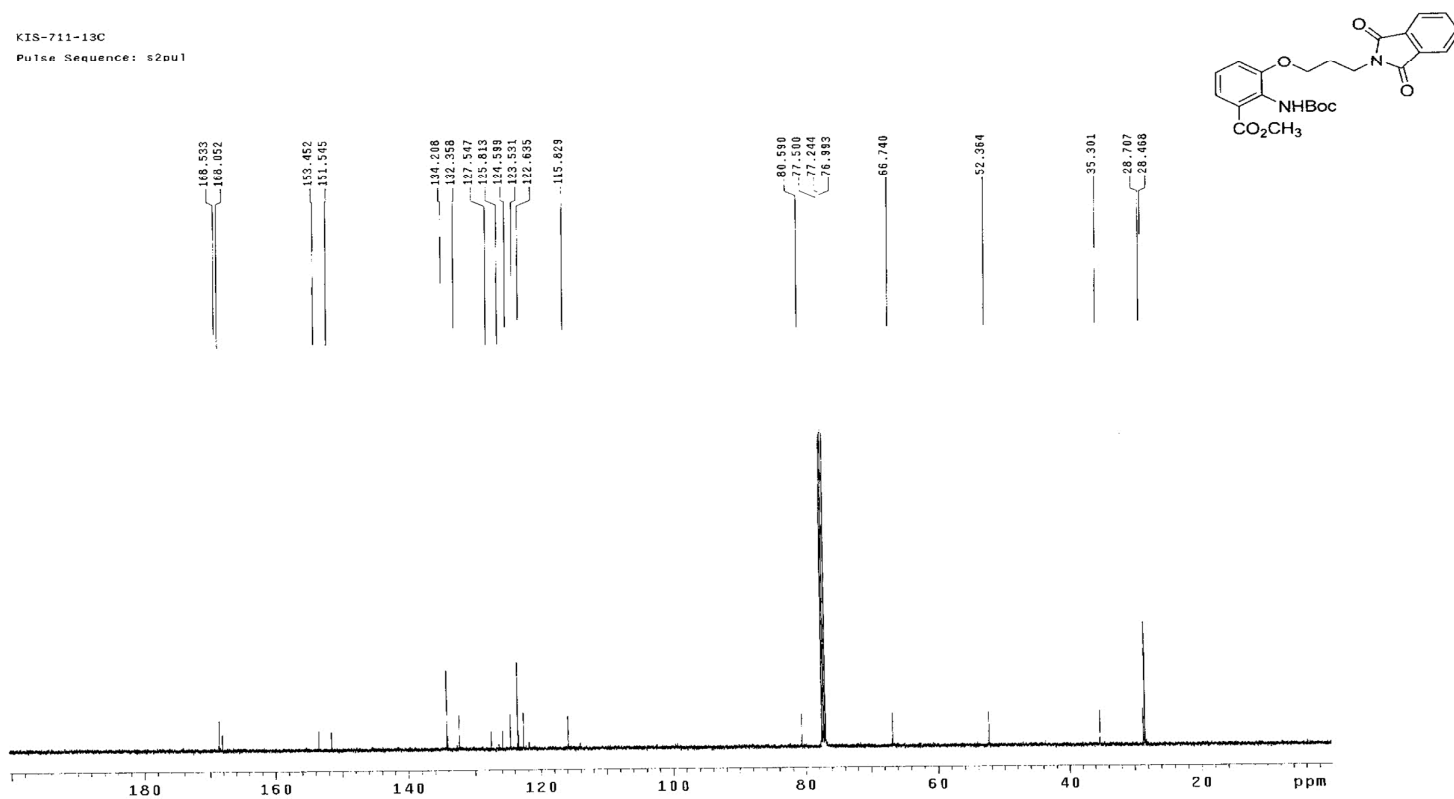


Figure S13. ¹³C NMR spectrum of compound **3** (CDCl₃, 125 MHz).

KIS 712
Pulse Sequence: s2pu1

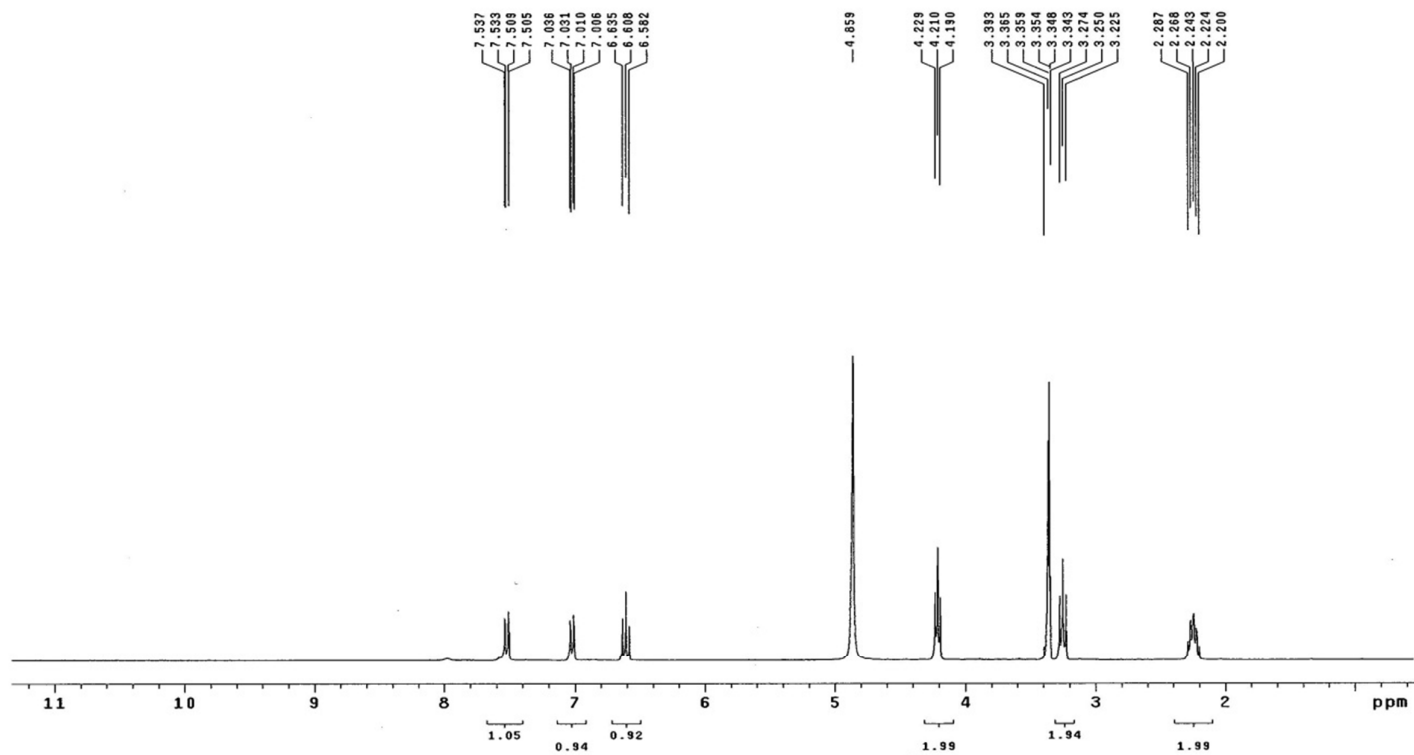


Figure S14. ^1H NMR spectrum of the synthesized compound **1** (CD_3OD , 300 MHz).

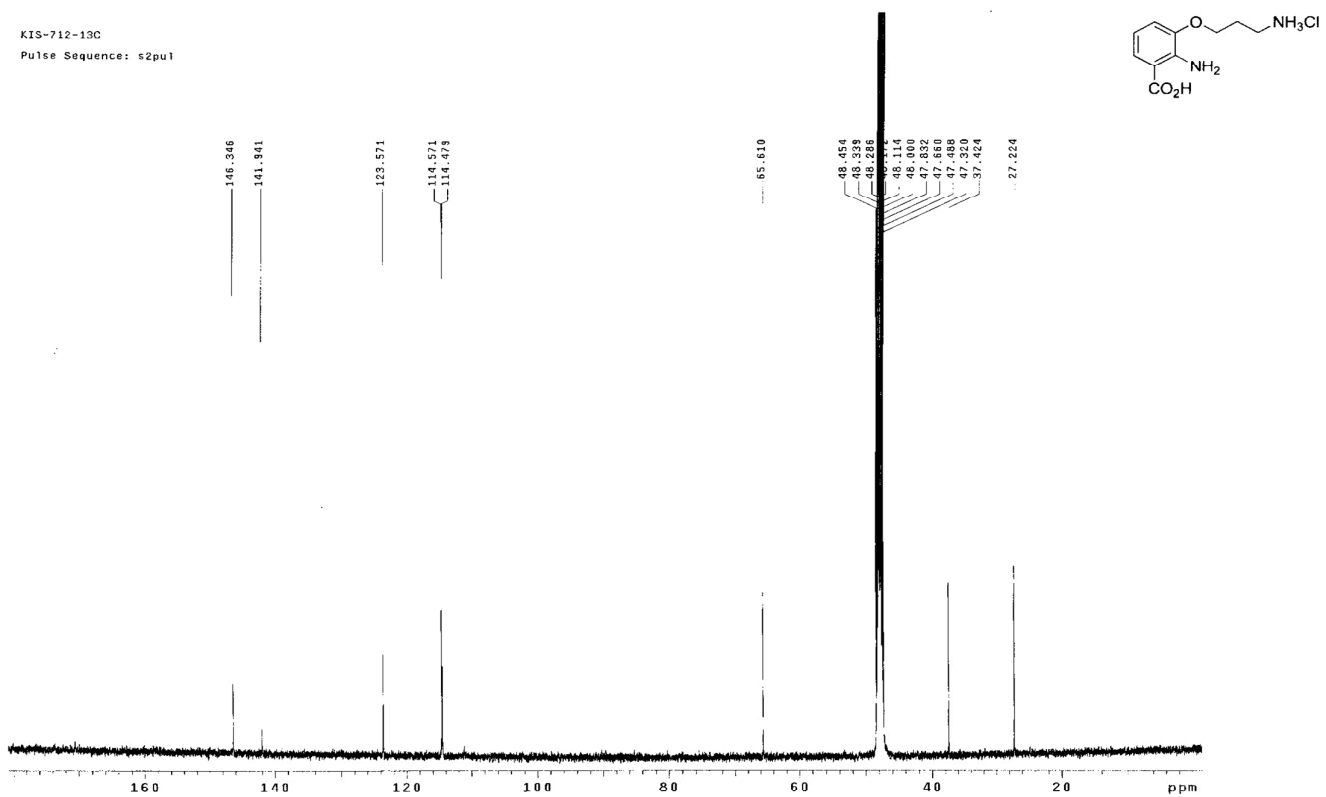


Figure S15. ^{13}C NMR spectrum of the synthesized compound 1 (CD_3OD , 125 MHz).

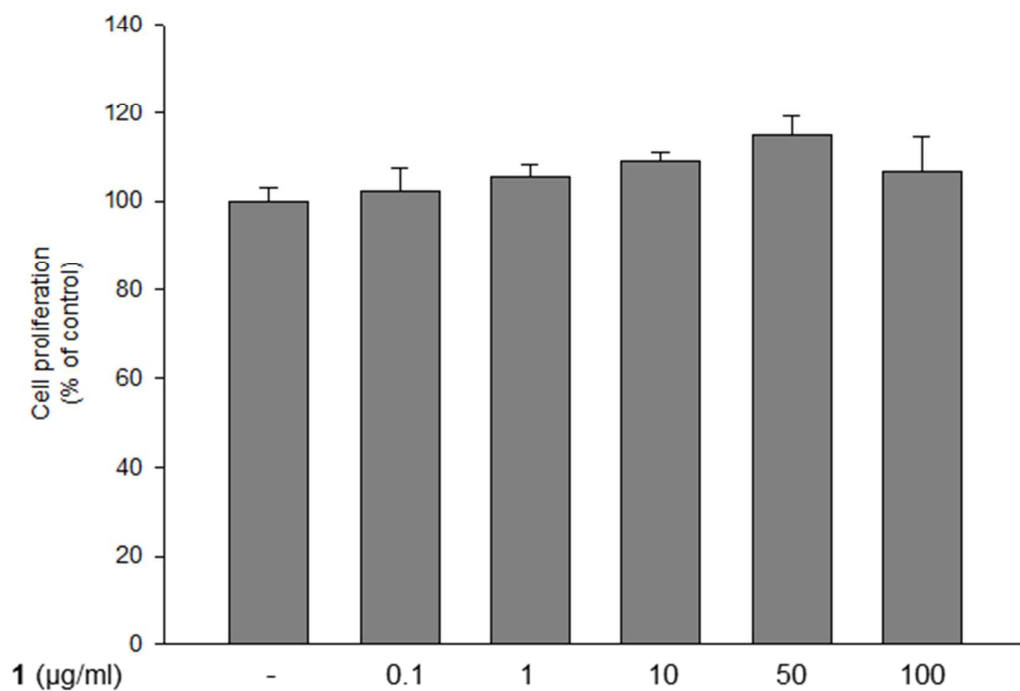


Figure S16. Effect of compound **1** on cell proliferation. RAW 264.7 cells were treated with **1** (0.1, 1, 10, 50, 100 µg/mL) for 24 h and cell proliferation was determined by the MTT assay. The data shown represent percentages of viable cells compared to the untreated cells. The data are presented as mean \pm S.E.M. Results are representative of at least three independent experiments.