

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 S15. ^{13}C NMR spectrum of the synthesized compound 1 (CD₃OD, 125 MHz).

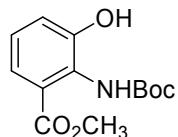
Figure S16. Effect of compound 1 on the cell proliferation.

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).



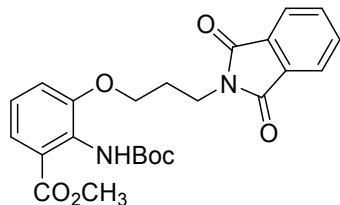
To a stirred solution of 3-hydroxyanthranilic acid (0.47 g, 3.069 mmol) in anhydrous MeOH (5.1 mL) was added SOCl_2 (0.11 mL, 1.228 mol) at 0 °C under N_2 . The reaction mixture was refluxed for 16 h. The resulting mixture was cooled in an ice bath, neutralized by the addition of solid NaHCO_3 (33 mg, 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.

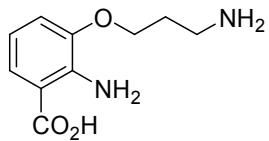
2.2. Methyl 2-(*tert*-butoxycarbonylamino)-3-[3-(1,3-dioxoisoindolin-2-yl)propoxy]benzoate (3).



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.

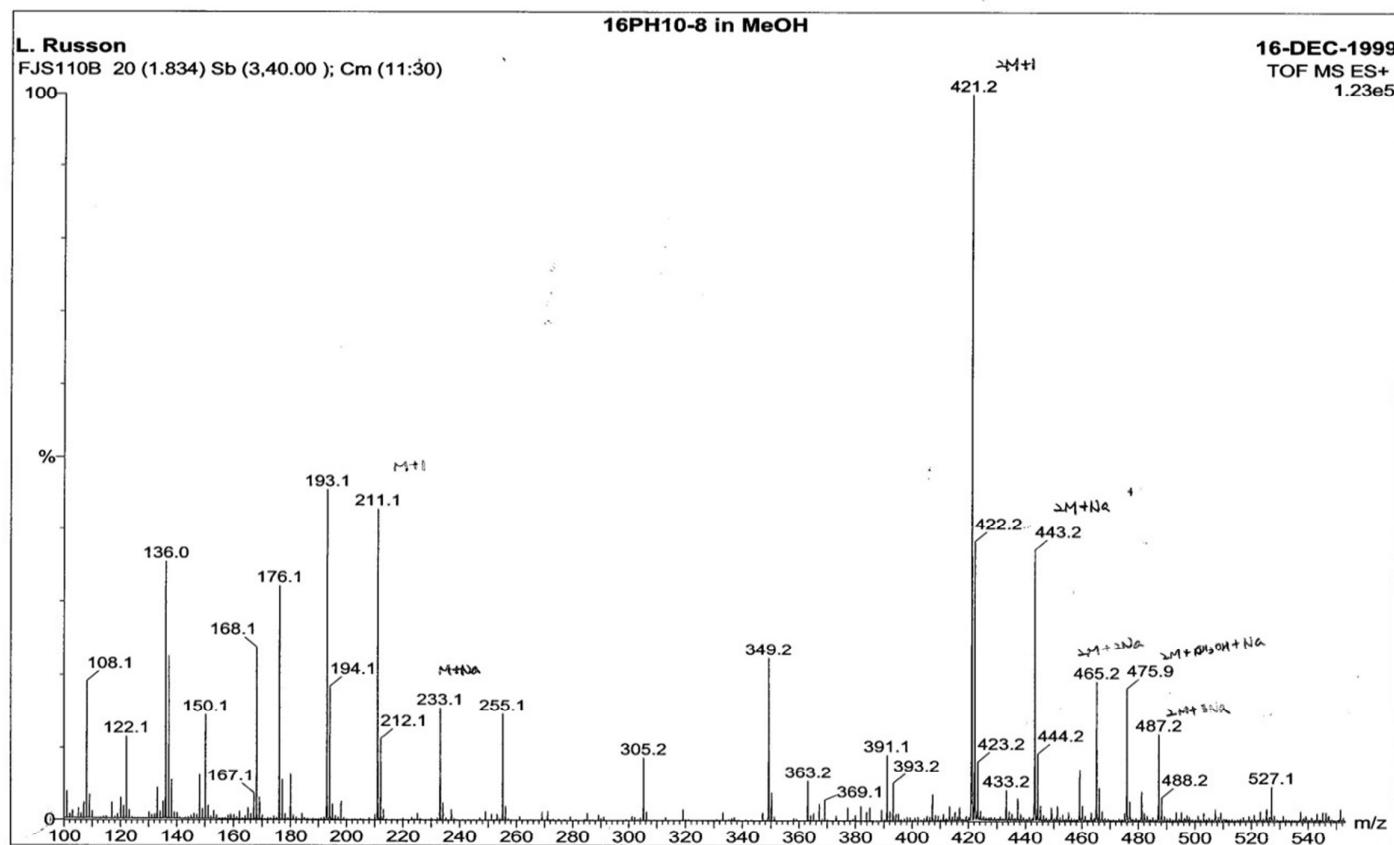


Figure S1. ESIMS spectrum of compound 1.

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 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

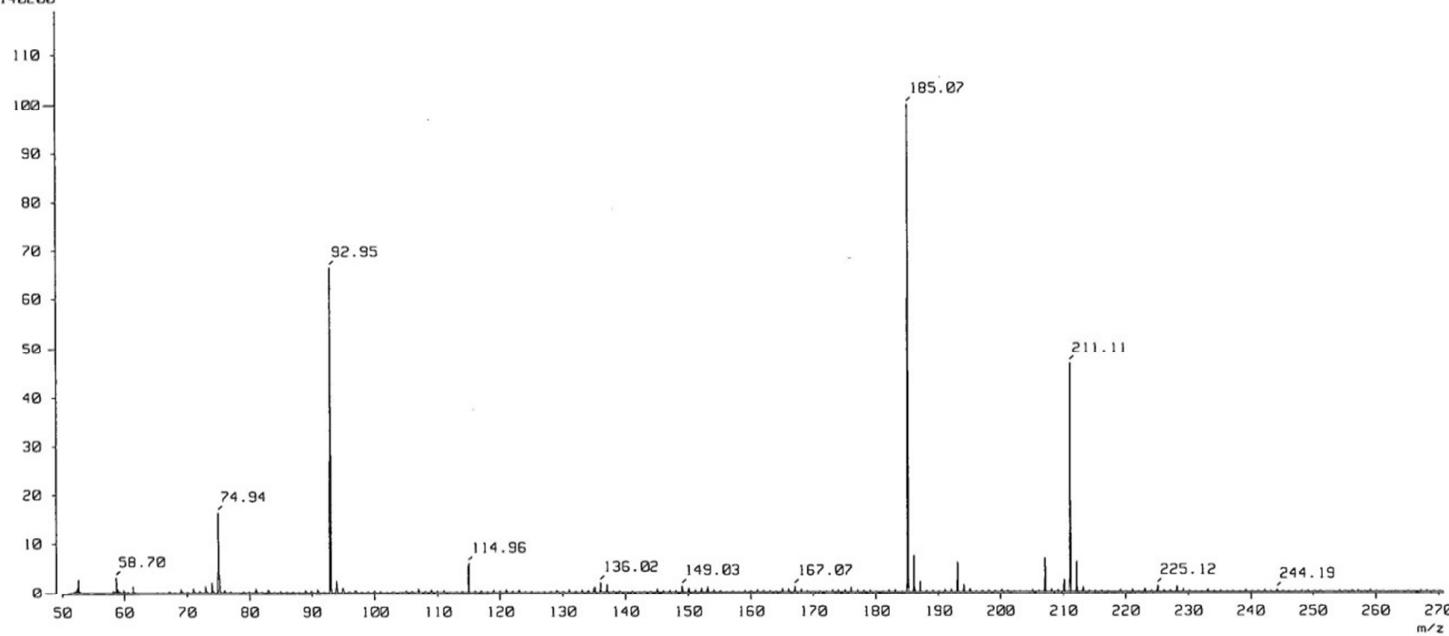


Figure S2. FABMS spectrum of compound 1.

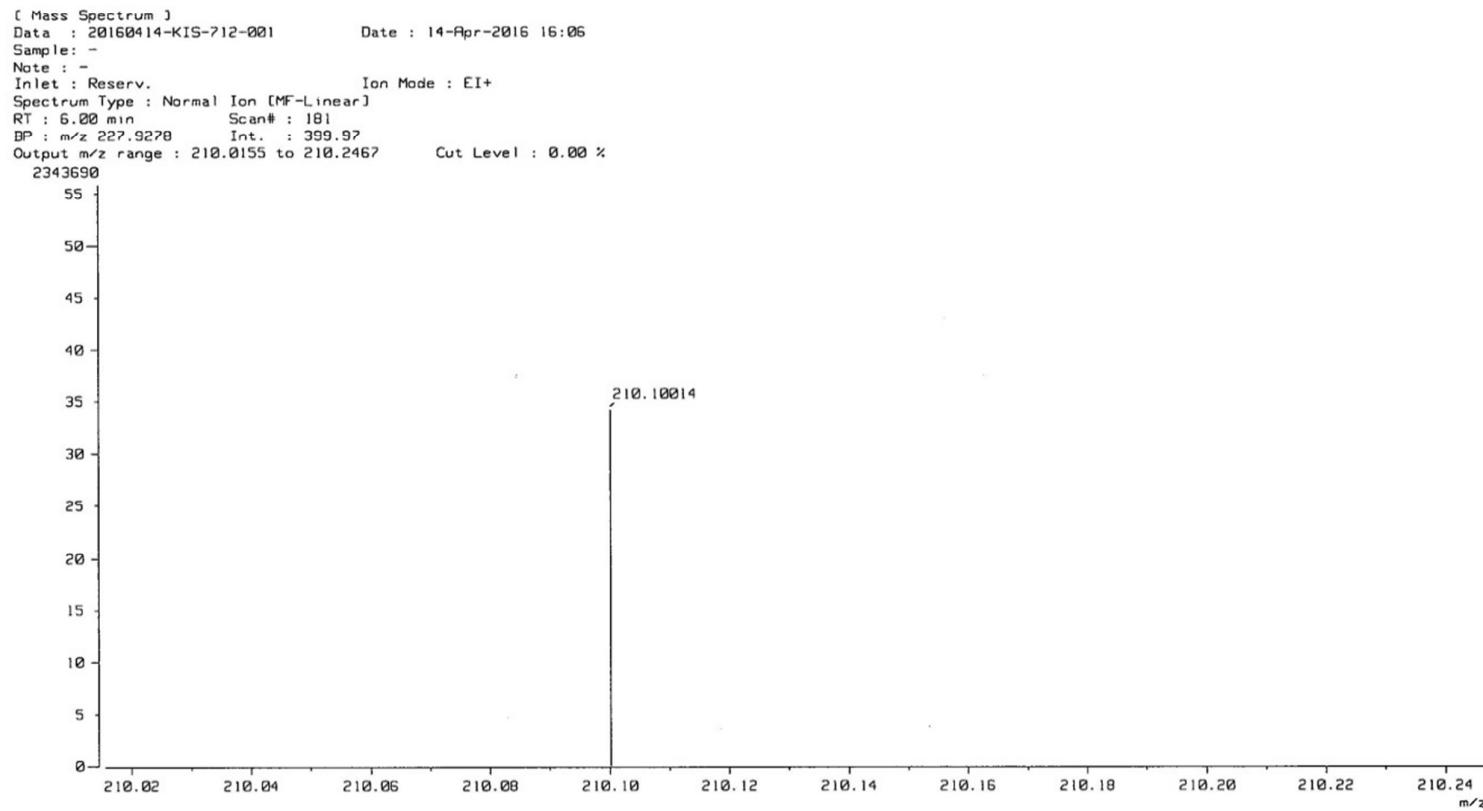


Figure S3. HREIMS spectrum of compound **1**.

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solvent  cd3od  dn   H1
file /export/home1/~ dpwr   30
/fjs/kwak/16Ph95/1/~ dof   0
/16Ph95/1/~ dnm   nnn
ACQUISITION      dnm   c
sfrq  499.883 dmf   200
tn    H1   dseq
at    1.892 dres   1.0
np    30072 homo   n
sw    8000.0  PROCESSING
fb    4400  wtf file
bs    32   proc   ft
tpwr  58   fn    not used
pdi   5.5  math   f
d1    0    werr
t0f   0    wexp
nt    8    wexp
ct    8    wbs
alock n    wnt   wft
gain  24

FLAGS
f1    n
in    y
dp    n
hs    nn

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wp    5515.0
vs    201
sc    5
wc    215
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rfp   1649.7
th    7
ins   1.000
nm   cdc ph

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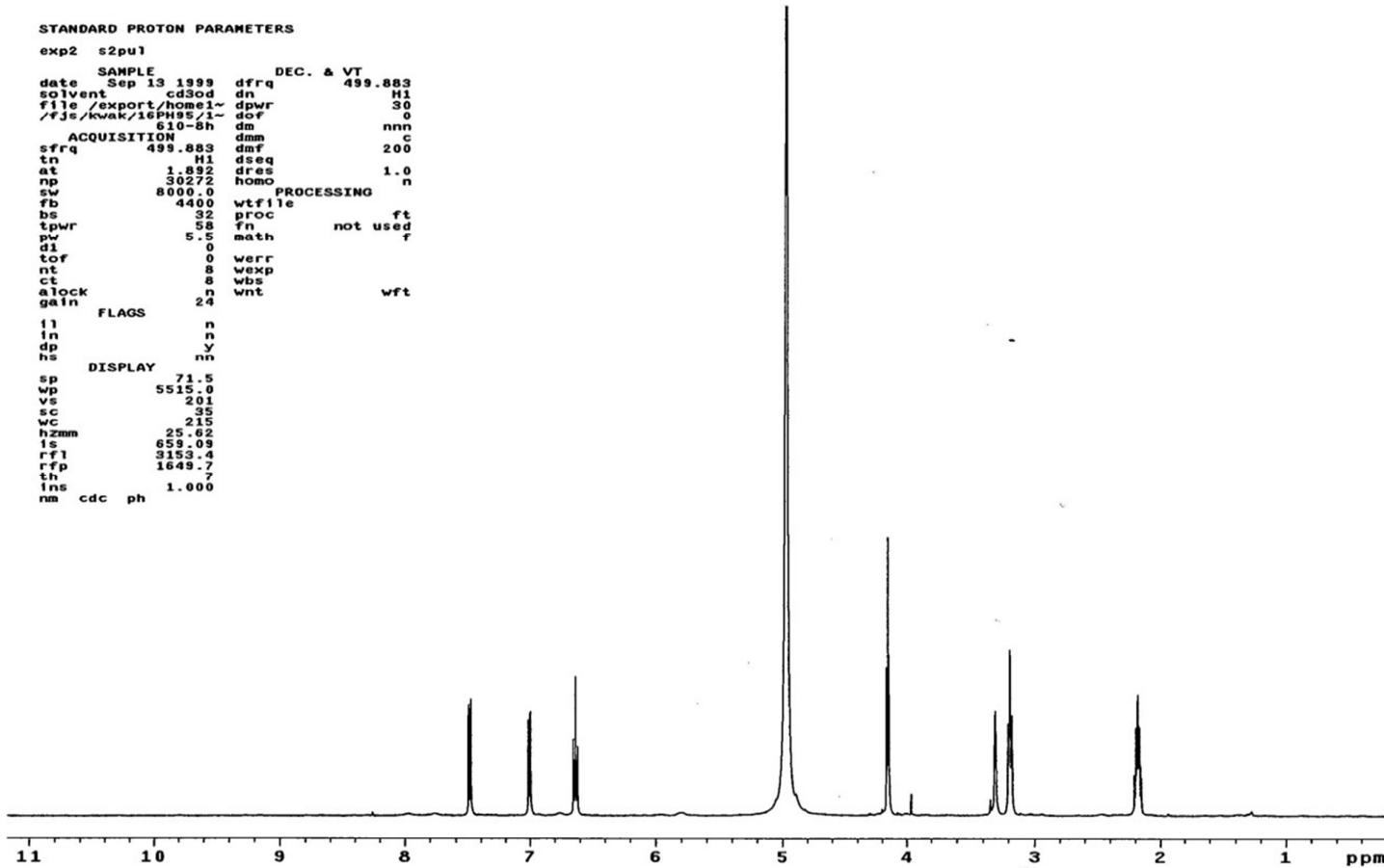


Figure S4. ^1H NMR spectrum of compound **1** (CD_3OD , 500 MHz).

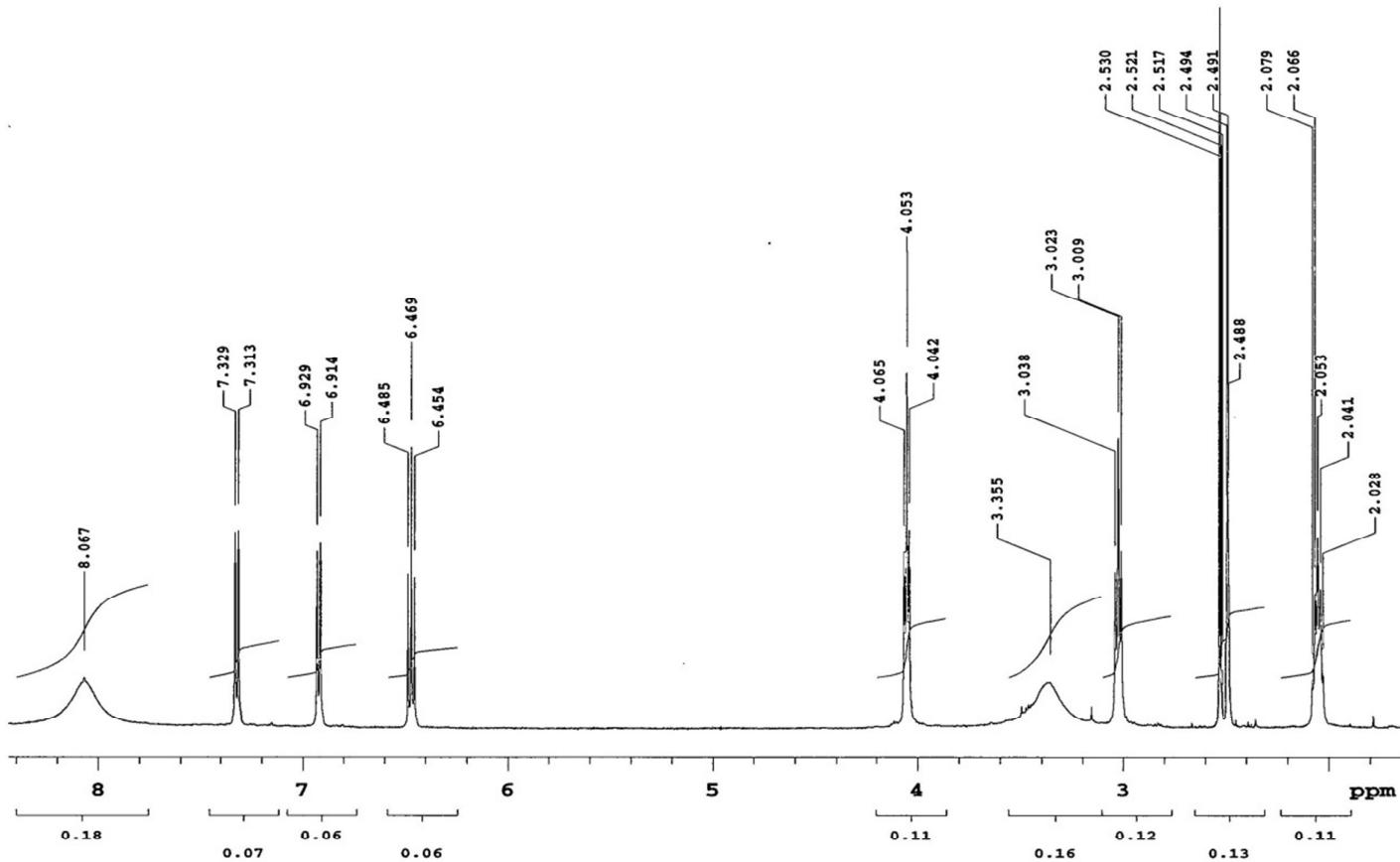


Figure S5. ^1H NMR spectrum of compound 1 (DMSO- d_6 , 500 MHz).

STANDARD CARBON PARAMETERS

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solvent	cd3od	dn
file	/export/home1~	dpwr
/fjs/kwak/16PH95/1~	dof	28
	elio-8c	0
ACQUISITION	dm	yyy
sfrq	125.707	dmf
tn	C13	dseq
at	0.999	dres
np	58368	homo
sw	30075.5	PROCESSING
fb	16500	lb
bs	32	wtflic
tpwr	54	proc
pw	3.1	ft
dd	1.000	not used
tof	0	math
nt	10000	werr
ct	10000	wexp
alock	n	wbs
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FLAGS		
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in	n	
dp	y	
hs	nn	
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wp	25355.3	
vs	362	
sc	0	
wc	250	
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rfp	6159.3	
th	68	
ins	1.000	
nm	cdc	ph

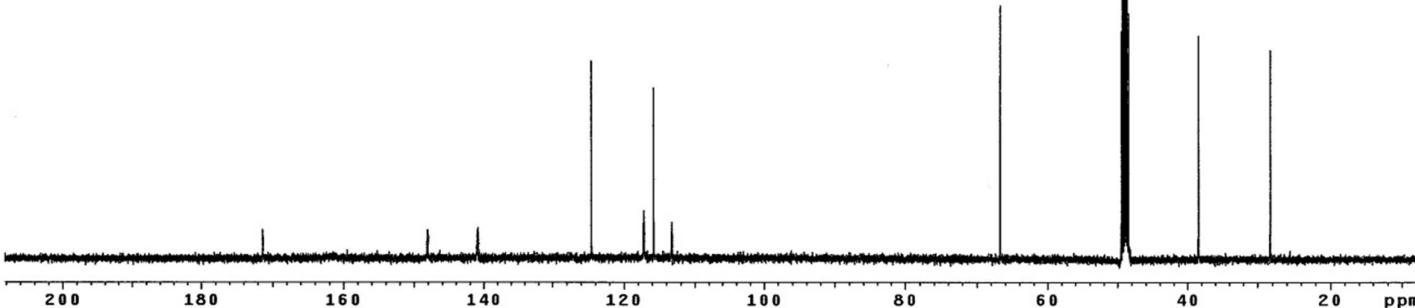


Figure S6. ^{13}C NMR spectrum of compound **1** (CD_3OD , 125 MHz).

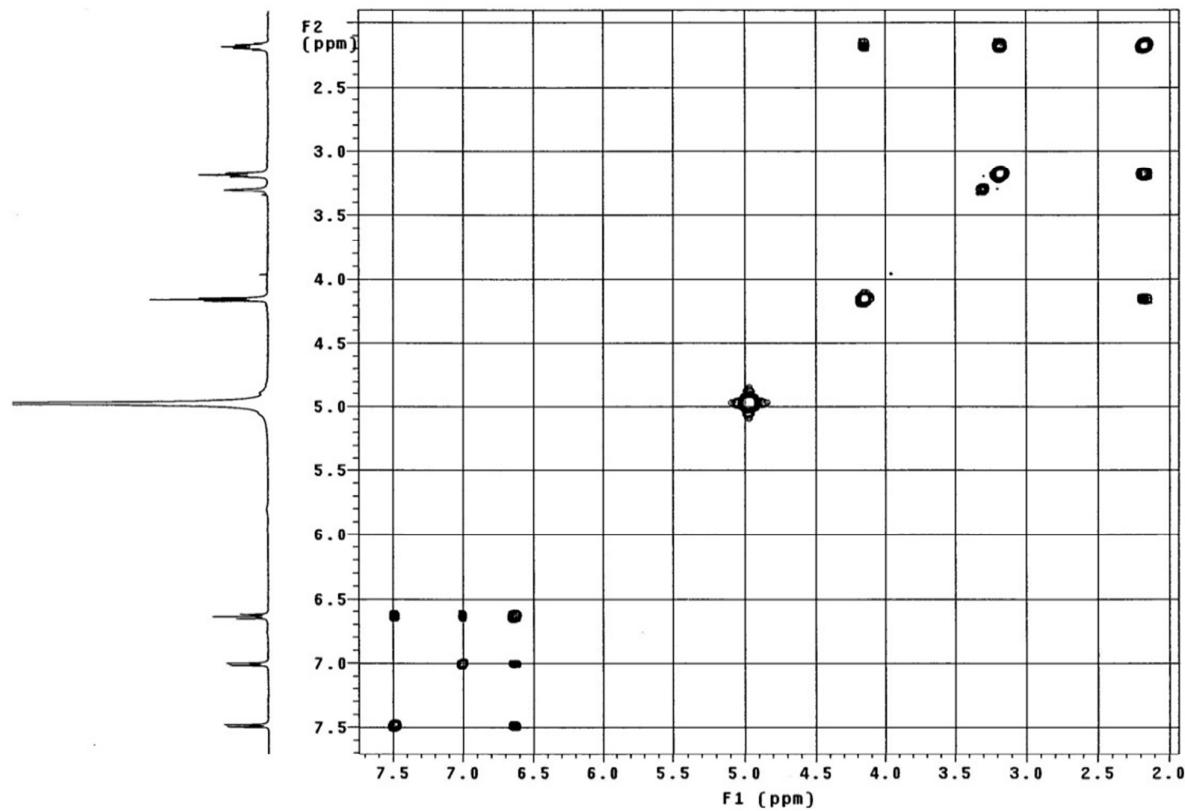


Figure S7. ^1H - ^1H COSY spectrum of compound **1** (CD_3OD , 500 MHz).

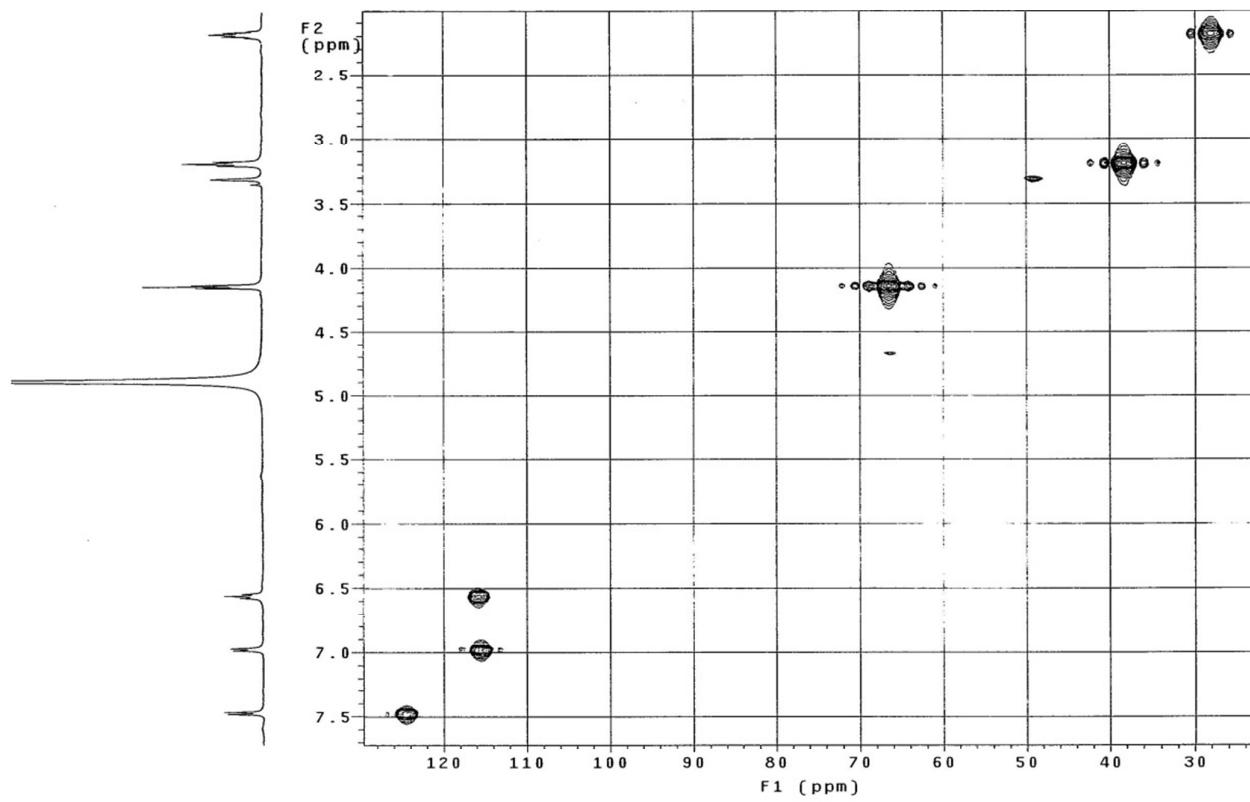


Figure S8. HMQC spectrum of compound **1** (CD_3OD , 500 MHz).

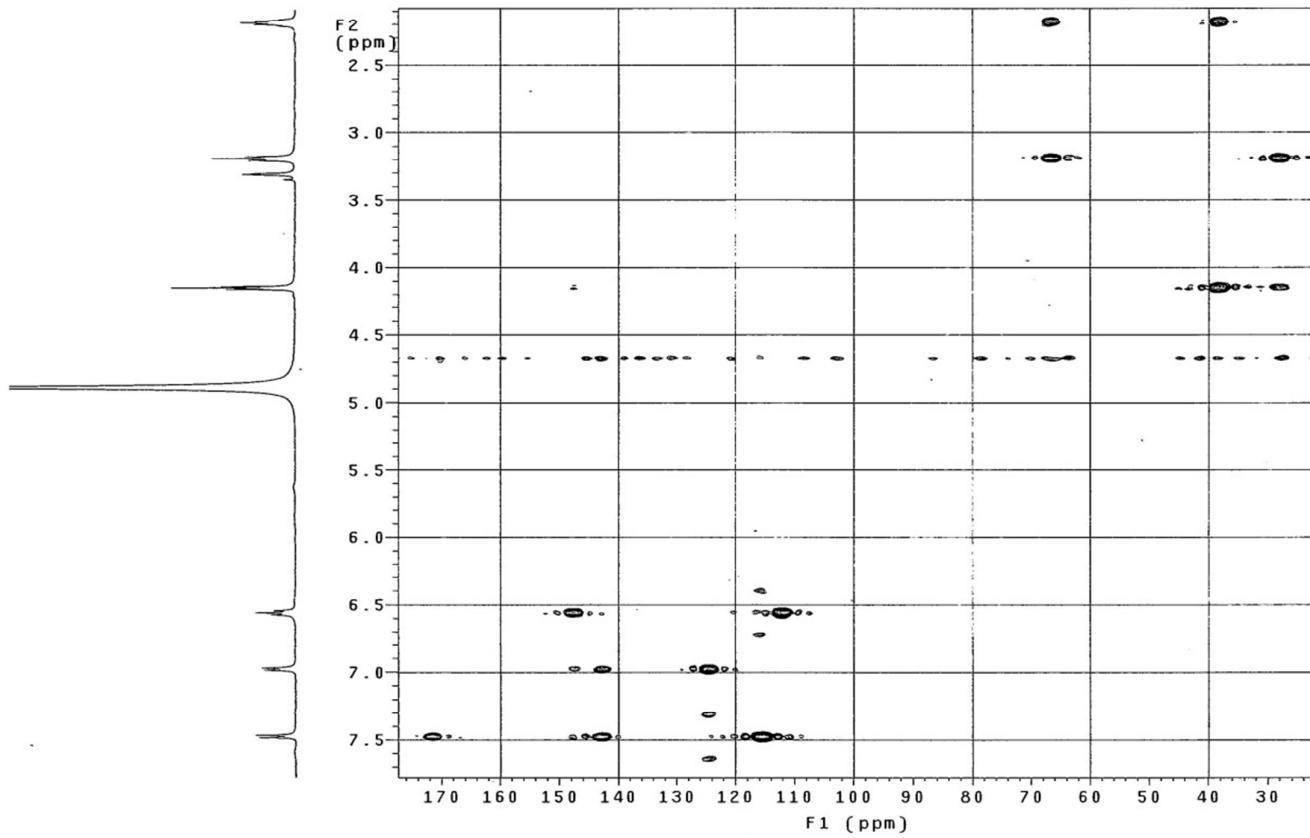
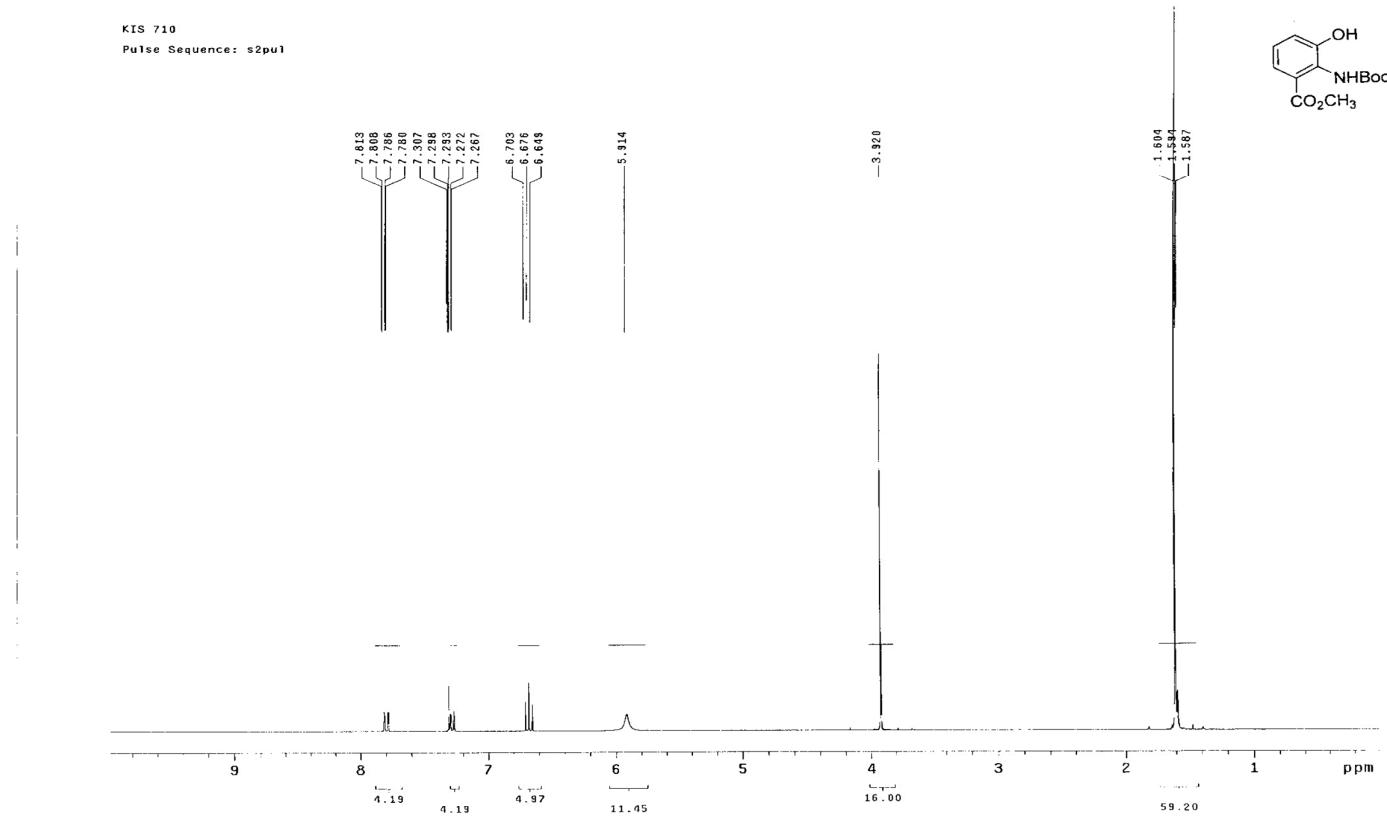


Figure S9. HMBC spectrum of compound **1** (CD_3OD , 500 MHz).



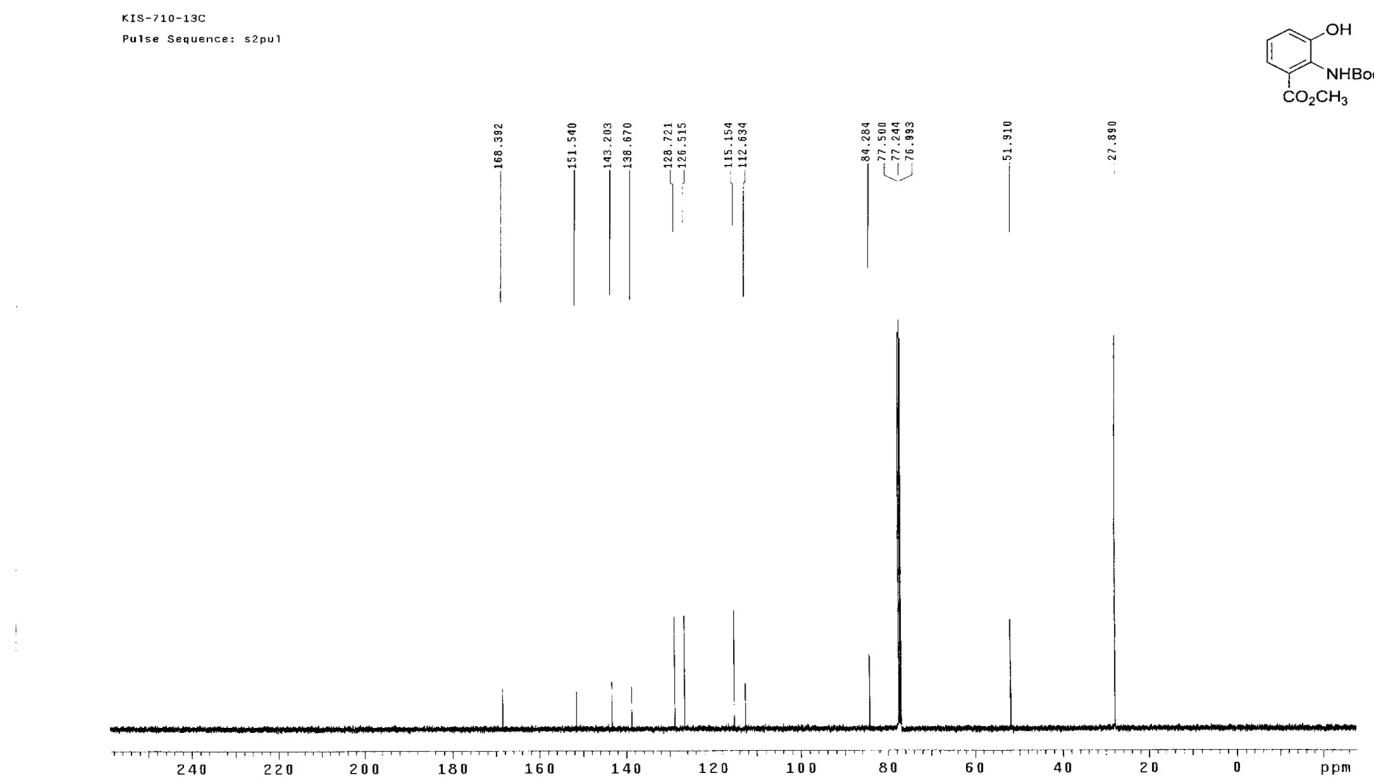


Figure S11. ^{13}C NMR spectrum of compound 2 (CDCl_3 , 125 MHz).

KIS 711
Pulse Sequence: s2pul

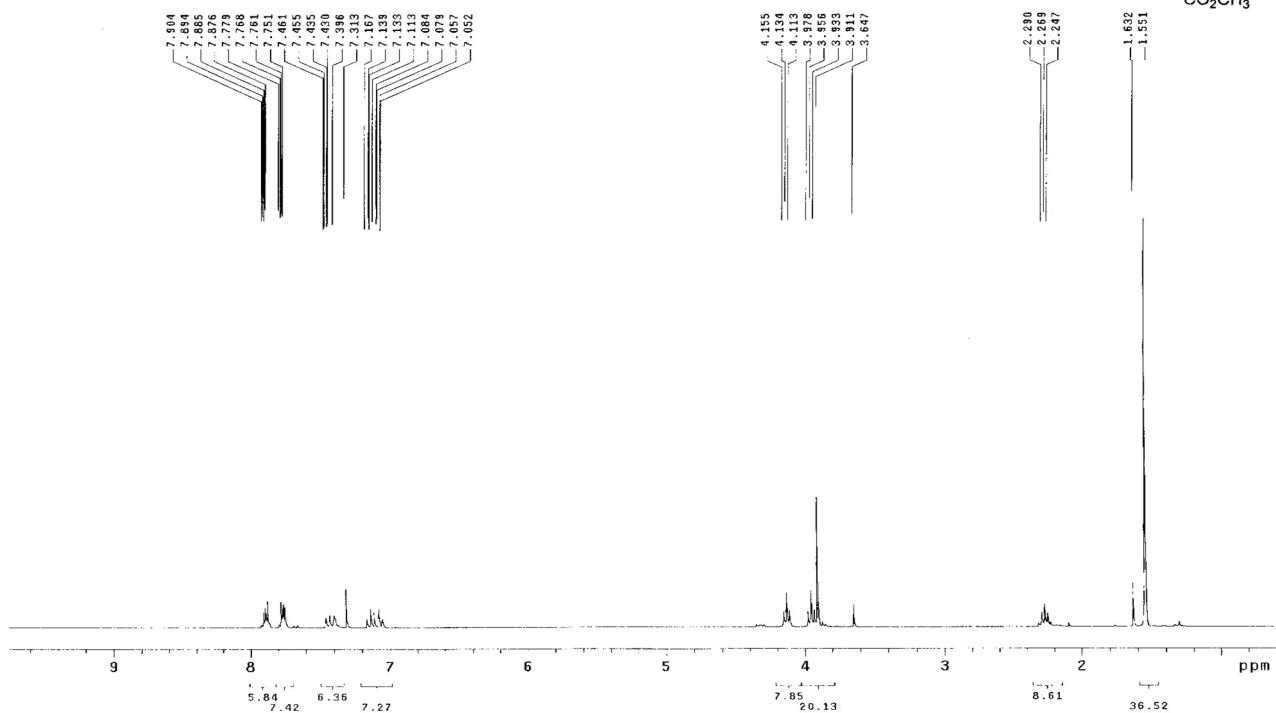


Figure S12. ^1H NMR spectrum of compound 3 (CDCl_3 , 300 MHz).

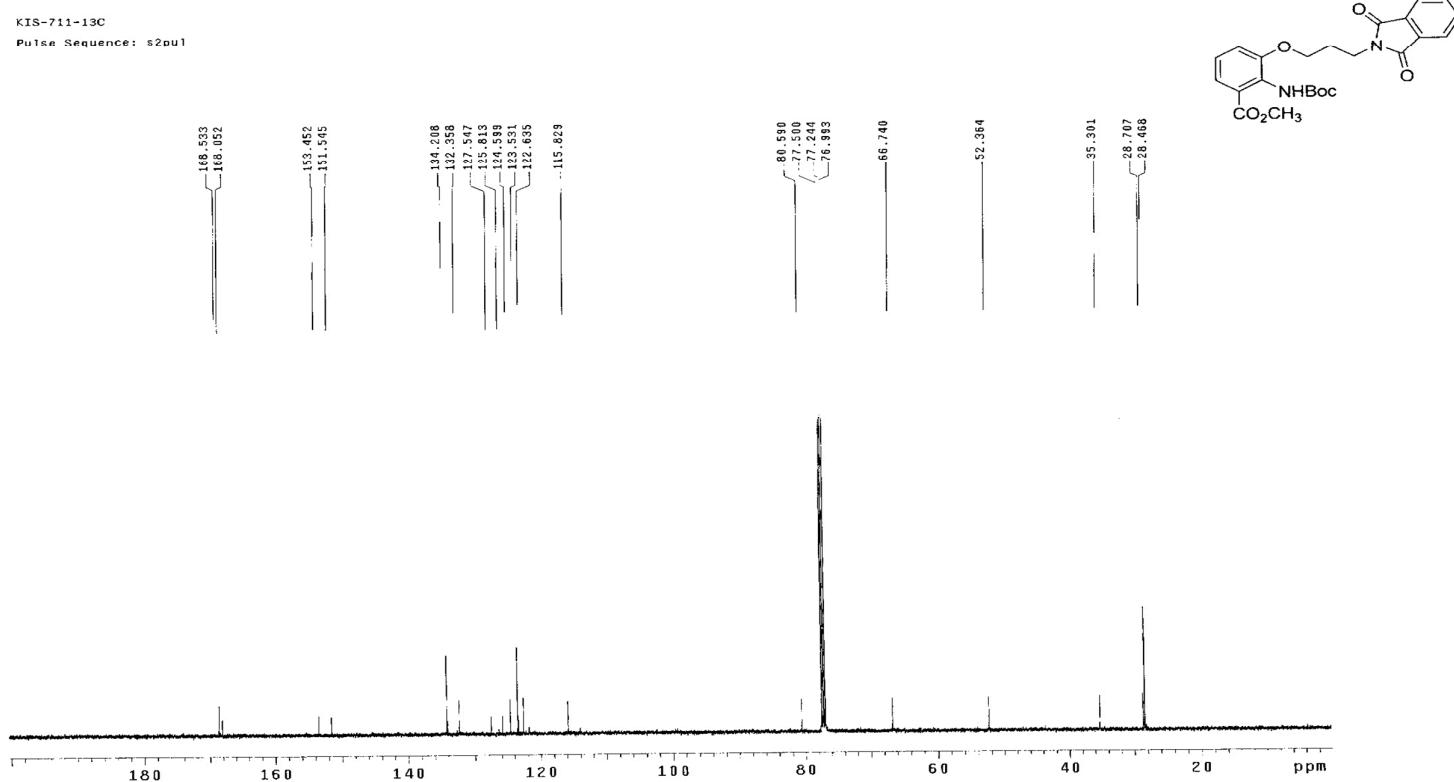


Figure S13. ^{13}C NMR spectrum of compound **3** (CDCl_3 , 125 MHz).

KIS 712
Pulse Sequence: s2pul

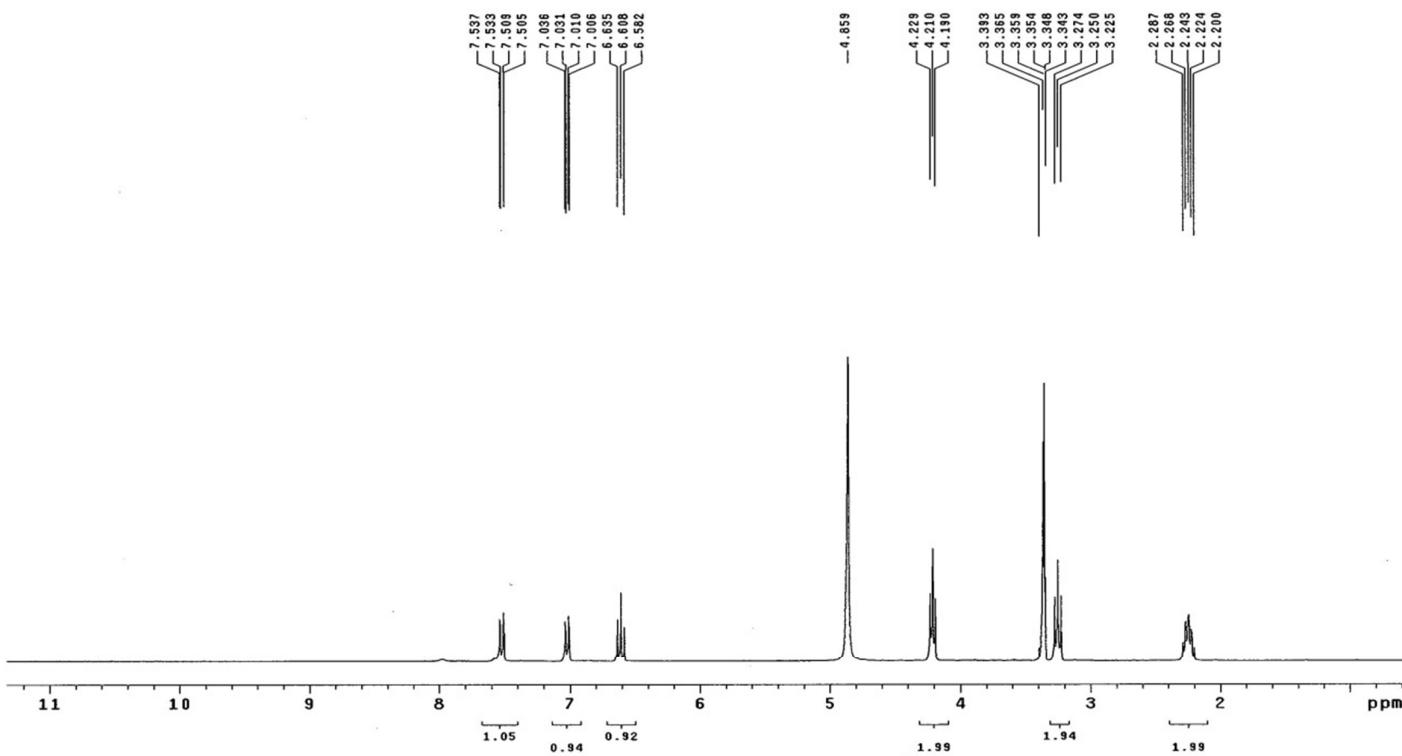


Figure S14. ^1H NMR spectrum of the synthesized compound **1** (CD_3OD , 300 MHz).

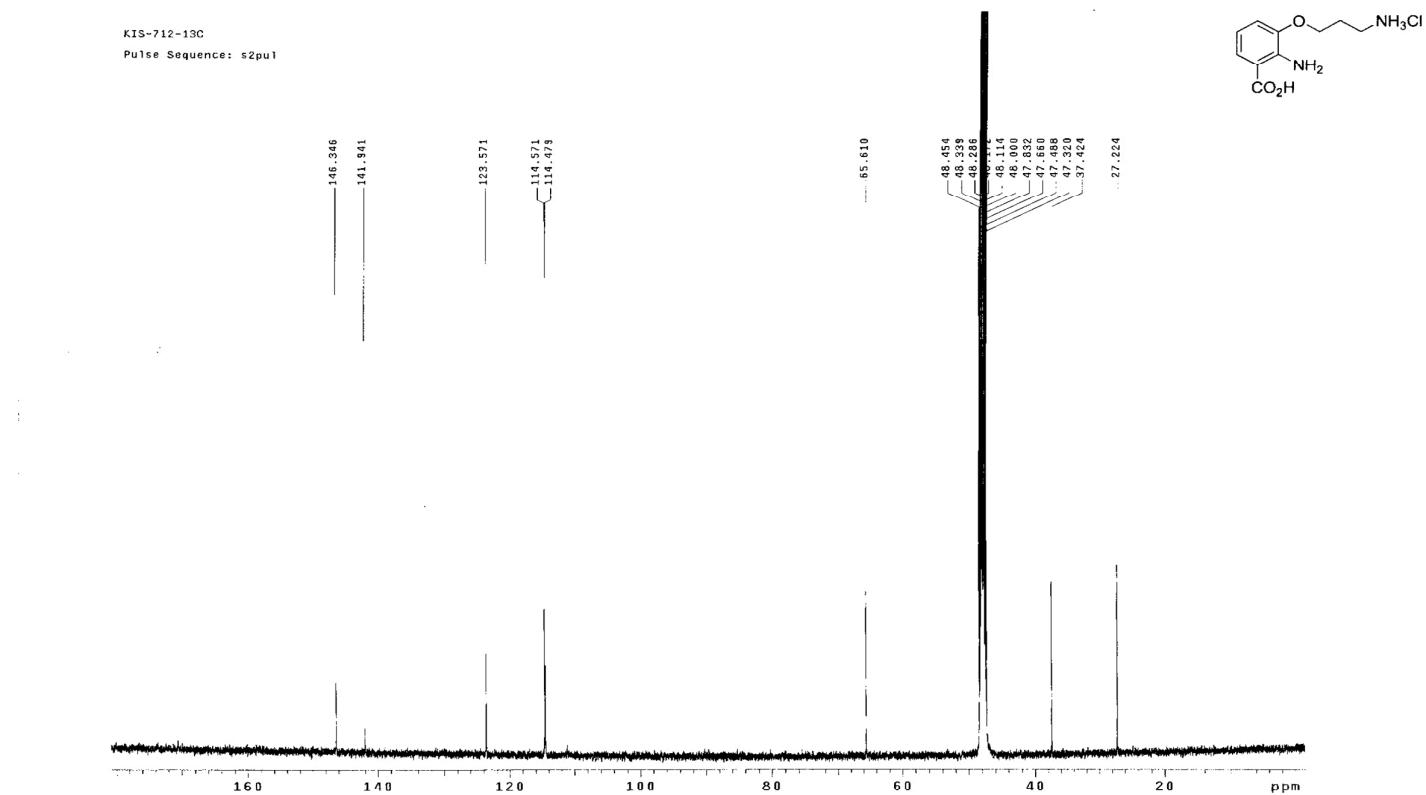


Figure S15. ^{13}C NMR spectrum of the synthesized compound **1** (CD_3OD , 125 MHz).

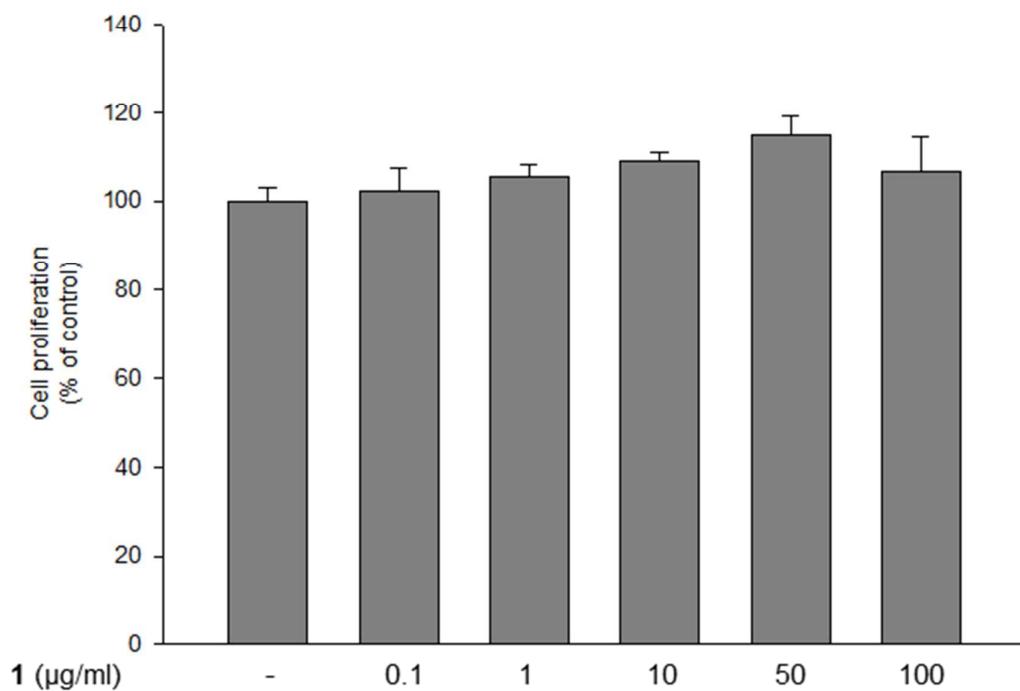


Figure S16. Effect of compound **1** on cell proliferation. RAW 264.7 cells were treated with **1** (0.1, 1, 10, 50, 100 μ g/mL) for 24 h and cell proliferation was determined by the MTT assay. The data shown represent percentages of viable cells compared to the untreated cells. The data are presented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