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Supporting Information

Acetogenin and Prenylated Flavonoids from *Helminthostachys zeylanica* with Inhibitory Activity on Superoxide Generation and Elastase Release by Neutrophils

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Measurement of rat mast cell degranulation and neutrophil degranulation [1-5]

A compound stock solution (30 μg/mL in DMSO) was prepared and stored at -25 °C. It was then diluted with DMSO to 1-30 μg/mL range at room temperature before the experiment. The final percentage of DMSO in the reaction mixture was less than 0.5% (v/v). Rat (Sprague Dawley, 250-300 g) peritoneal mast cells and peripheral blood neutrophils were isolated and incubated with test compounds for 5 min at 37 °C before stimulation with 10 μg/mL of the compound 48/80 for another 15 min or with 1 μM FMLP/CB for another 45 min, respectively. The degranulation of mast cells and neutrophils was assessed by the determination of histamine and β-glucuronidase, as

well as β-glucuronidase and lysozyme in the supernatant, respectively. The total content of lysozyme and β-glucuronidase was measured from the *Micrococus lysodeikticus*-treated cells and phosphoglucuronide.

1 Wang JP, Raung SL, Kuo YH and Teng CM. Daphnoretin-induced respiratory burst in rat neutrophil is probably mainly through protein kinase C activation. Eur J Pharmacol 1995; 288: 341-348

2 Barrett AJ. Lysosomes. In: Dingle JT, editor. A laboratory handbook. Amsterdam: Elsevier; 1972: 118-120

3 Absolom DR. Basic methods for the study of phagocytosis. Methods Enzymol 1986; 132: 92-179

4 Wang JP, Hsu MF, Ouyang C, Teng CM. Edematous response caused by[Thi^{5,8} D-Phe]bradykinin, $\alpha\beta_2$ receptor antagonist, is due to mast cell degranulation. Eur J Pharmacol 1989; 161: 143-149

5 Hakanson R, Ronnberg AL. Improved fluorometric assay of histamine. Analyt Biochem 1974; 60: 560-567

Table 1S UV spectral properties of compounds **2-5**

Compounds	λ_{max}^{MeOH} (nm)		$\lambda_{max}^{MeOH+AlCl_3}$ (nm)		$\lambda_{max}^{MeOH+AlCl_3+HCl}$ (nm)	
	Bd II	Bd I	Bd II	Bd I	Bd II	Bd I
2	271	349	275	333, 425	283	361
3	271	331	274	359, 465	273	340, 415
4	292	344	292	418	296,	350
5	251	300, 351	264	418	264	334, 393

Table 2S Inhibitory effects of crude extracts from *H. zeylanica* on β-glucuronidase and histamine by rat mast cell in response to compound 48/80

Drugs (μg/mL)		Percent Release	
		β-glucuronides (μg/mL) or (Inh %)	histamine (μg/mL) or (Inh %)
Control		14.2 ± 1.6	83.1 ± 2.2
Methanol extract	10	14.8 ± 1.0 (3.8 ± 8.9)	85.8 ± 7.9 (-3.0 ± 9.4)
	30	11.6 ± 0.9 (24.5 ± 6.3) *	76.3 ± 3.9 (8.3 ± 4.6)
n-Hexane extract	10	12.5 ± 1.3 (15.3 ± 4.4) **	92.3 ± 3.3 (-10.8 ± 4.1)
	30	13.3 ± 0.7 (12.9 ± 8.8)	93.2 ± 3.6 (-11.9 ± 4.6)
Chloroform extract	10	12.5 ± 0.5 (-6.1 ± 9.5)	81.6 ± 5.8 (-1.6 ± 6.6)
	30	7.8 ± 0.3 (33.4 ± 6.4) *	53.4 ± 0.3 (33.2 ± 3.0) **
Water extract	10	13.0 ± 1.7 (16.7 ± 6.9)	89.1 ± 1.5 (-7.0 ± 1.9)
	30	8.9 ± 1.5 (43.5 ± 6.6) **	79.3 ± 2.3 (4.8 ± 2.1)
Mepacrine	10 μM	12.1 ± 0.4 (18.1 ± 2.5) *	64.1 ± 2.3 (21.3 ± 2.5) *
	30 μM	7.2 ± 0.2 (46.3 ± 3.2) **	34.8 ± 1.1 (53.7 ± 1.3) **
	100 μM	3.1 ± 0.2 (81.9 ± 1.4) **	14.2 ± 1.3 (85.2 ± 1.7) **
	IC_{50}	27 ± 9.2	26.2 ± 1.6

Mepacrine was used as positive control. Results are presented as mean ± S.E.M. (n = 3).* $p < 0.05$, ** $p < 0.01$, compared with the control value.

Table 3S Inhibitory effects of crude extracts from *H. zeylanica* on β-glucuronidase and lysozyme by rat neutrophils in response to FMLP/C

Drugs	β-glucuronides	lysozyme
	IC_{50} (μg/mL)	IC_{50} (μg/mL)
Control	30.0 ± 0.7	29.3 ± 1.4
Methanol extract	1.1 ± 0.1	N
n-Hexane extract	2.0 ± 0.7	9.0 ± 2.9
Chloroform extract	1.3 N 0.1	N
Trifluoperazine	9.8 ± 0.4	9.6 ± 0.4

Trifluoperazine (TFP) was used as positive control. Results are presented as mean ± S.E.M. (n = 3).* $p < 0.05$, ** $p < 0.01$, compared with the control value. Water extract was inactive.

Table 4S Cytotoxicity activities of crude extracts from *H. zeylanica*

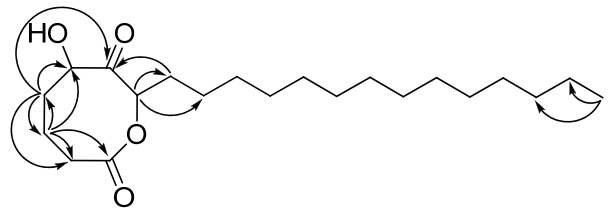
Compounds	HL-60[a]	U937[a]
	IC_{50} (μg/mL)[a] or (Inh %)[b]	IC_{50} (μg/mL)[a] or (Inh %)[b]
Methanol extract	19.9	21.0
n-Hexane extract	5.1	5.3
Chloroform extract	19.3	14.6
Ethyl acetate extract	7.8	5.9
Retinoic acid (0.03 μg/mL)	(34.2 ± 3.3)	(31.3 ± 1.3)

Retinoic acid (0.03 μg/mL) was used as positive control. Results are presented as mean ± S.E.M. (n = 3).

[a] HL-60 and U937 cells (2 x 10^4 cells) were incubated with different concentration in 96 hours.

[b] Percentage of inhibition (Inh %) at 0.03 μg/mL concentration.

Figure 1S Selected HMBC correlations of compounds **1-5.**



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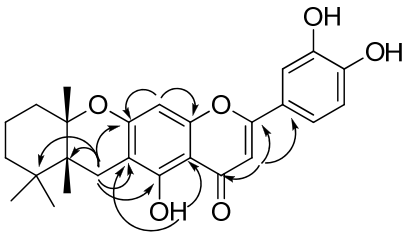

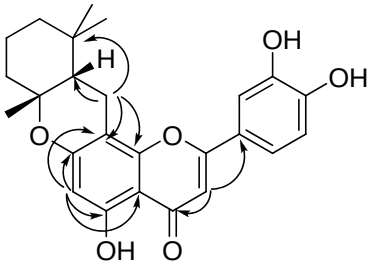

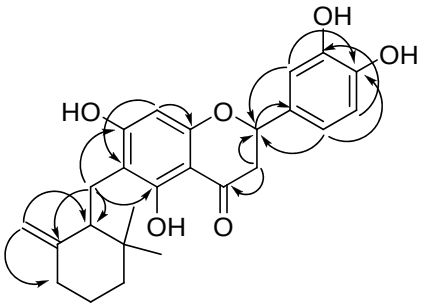

2 **3** **4**

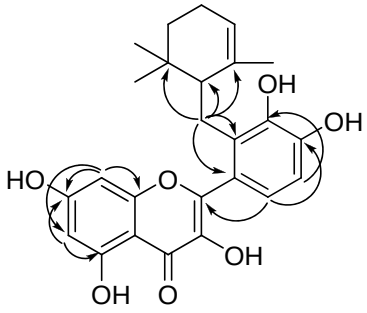

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Figure 2S The ^{1}H-NMR spectrums of ugonin L and compounds **2** and **3**.

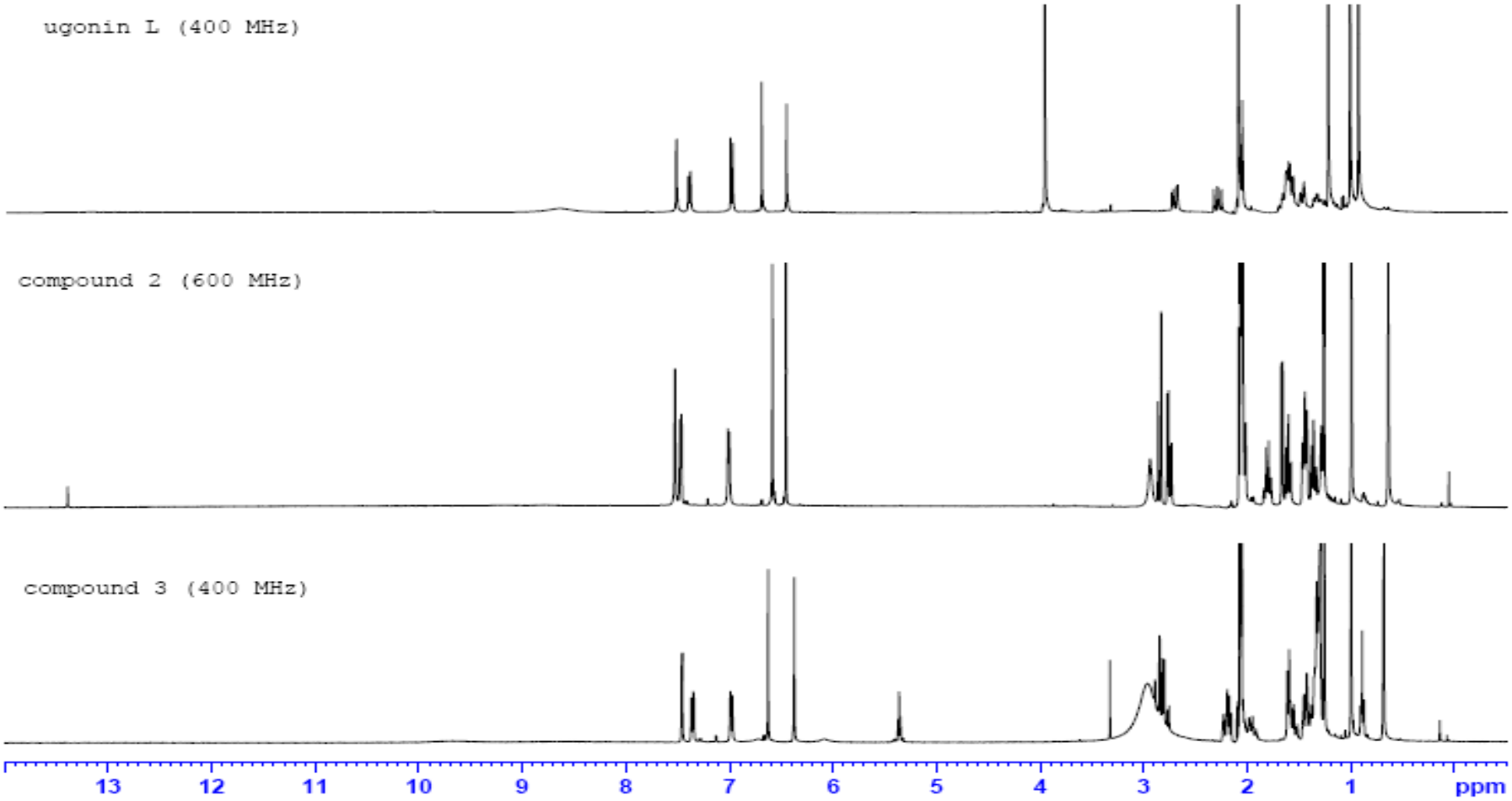


Figure 3S The ^{1}H-NMR spectrums of compound **2** measured in different *d*-solvents.

(1) Compound **2** measured in CD_3OD, 600 MHz

(2) Compound **2** measured in MeCO-d_6, 600 MHz

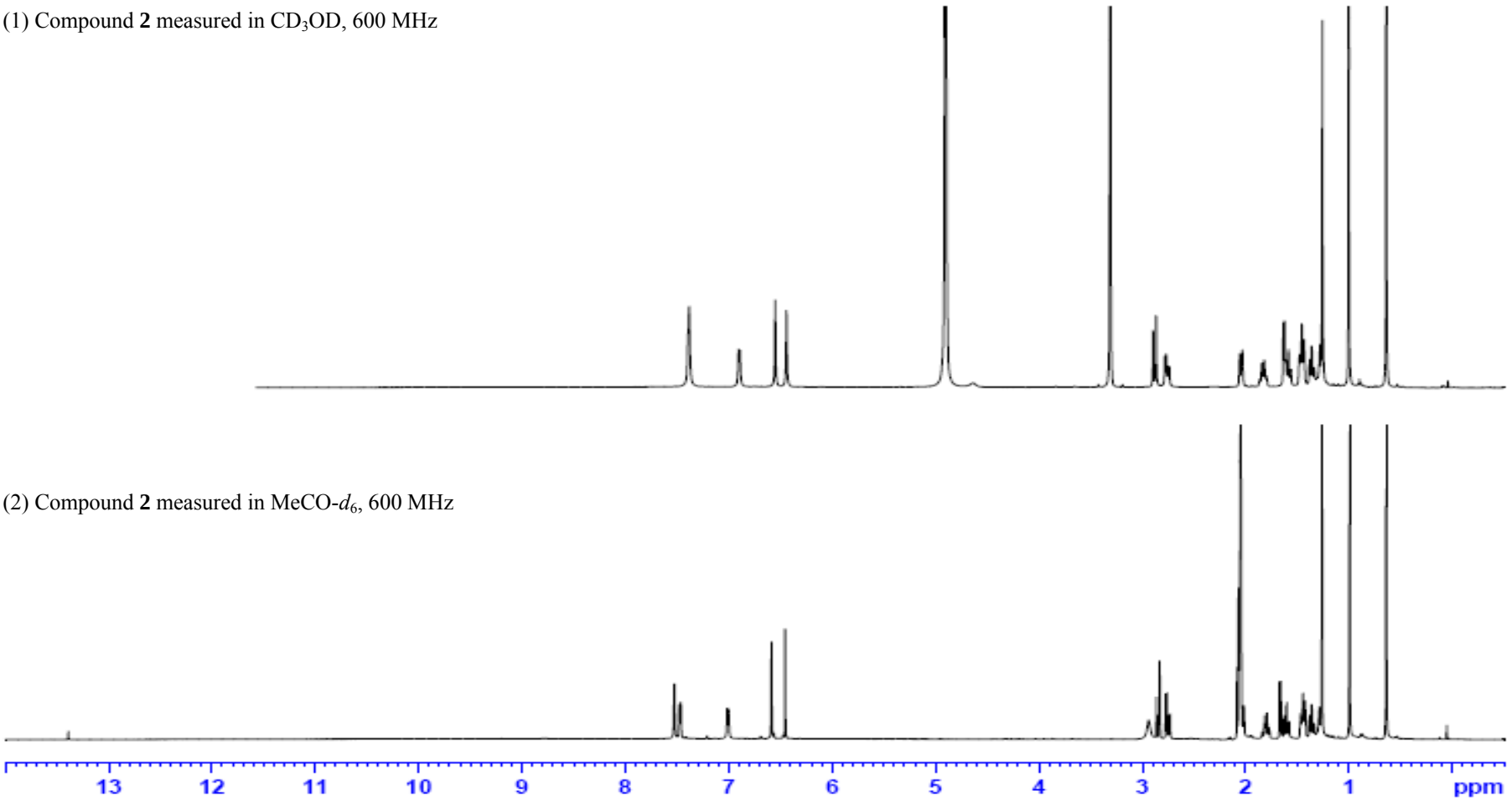


Figure 4S The ^{13}C-NMR spectrums of compound **2** measured in different *d*-solvents.

(1) Compound **2** measured in CD_3OD, 150 MHz

(2) Compound **2** measured in MeCO-d_6, 150 MHz

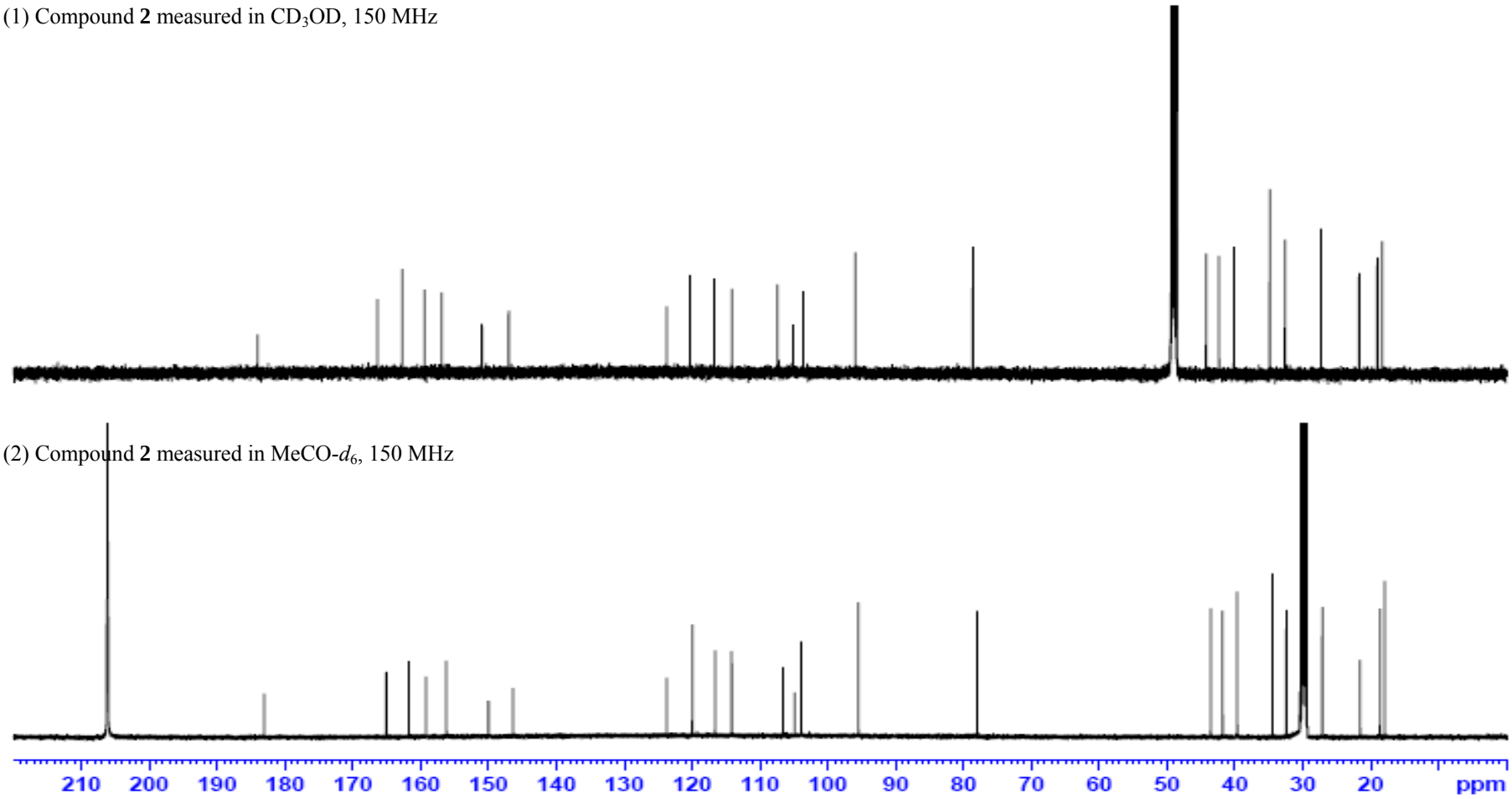
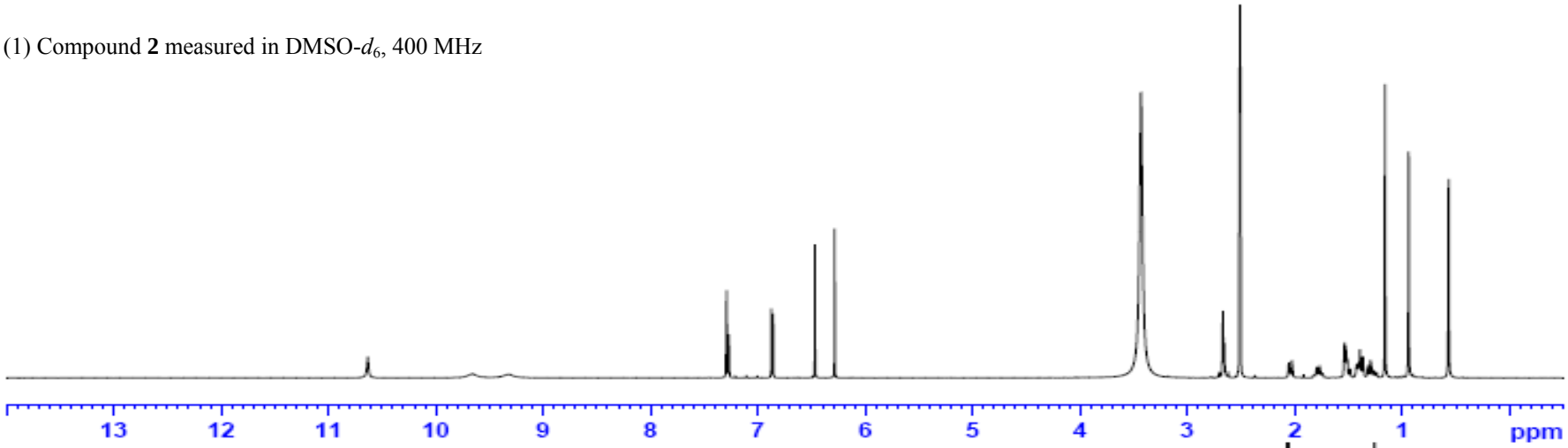


Figure 5S The ^{1}H-NMR spectrums of compound **3** measured in different *d*-solvents.

(1) Compound **2** measured in DMSO-d_6, 400 MHz



(2) Compound **3** measured in MeCO-d_6, 400 MHz

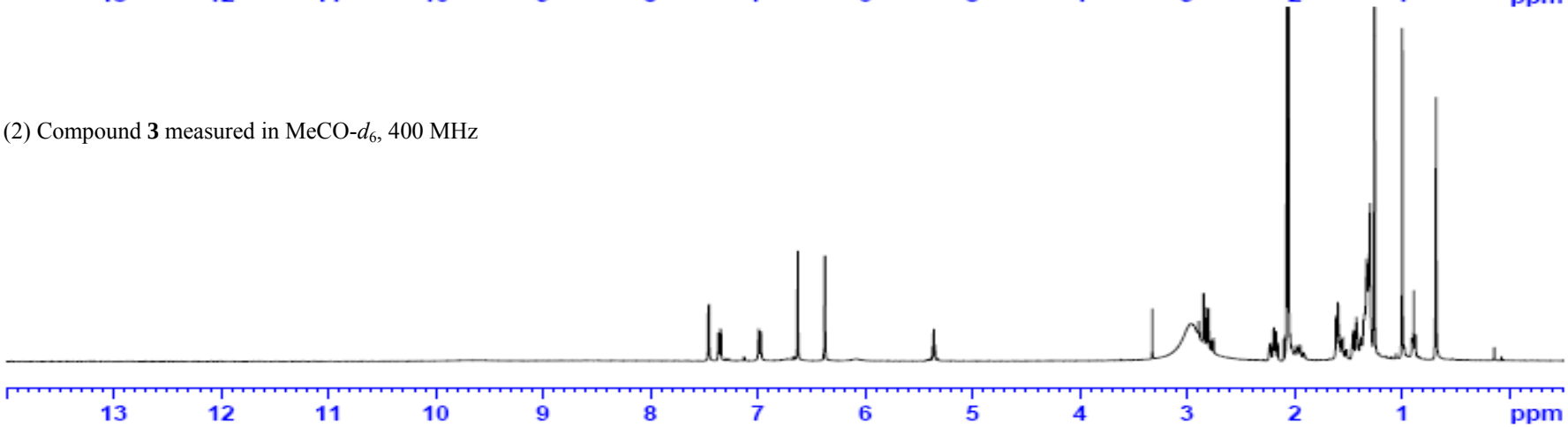
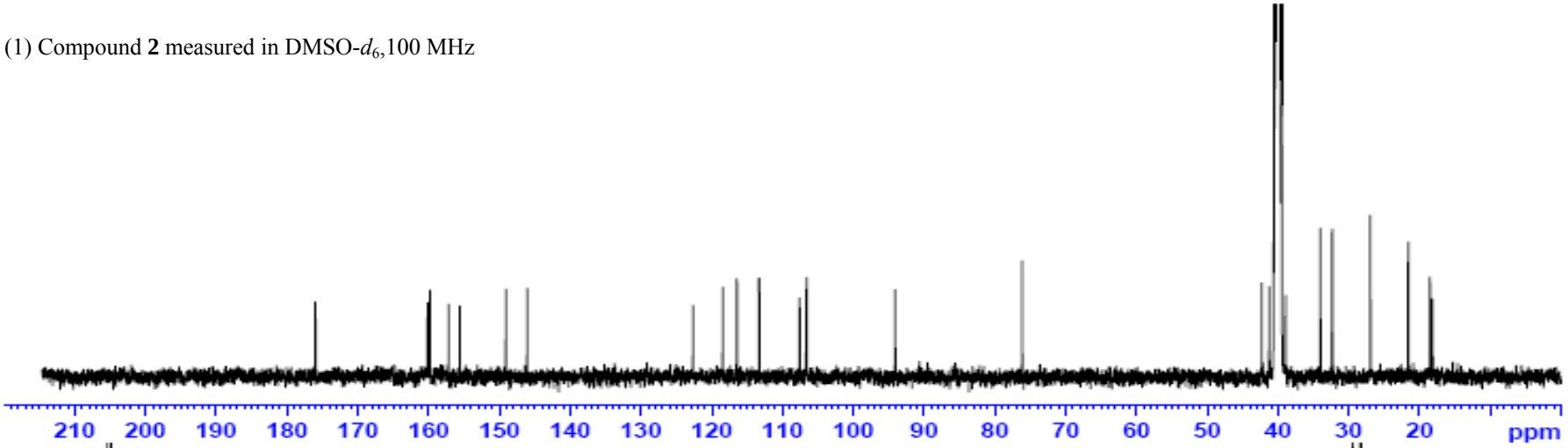


Figure 6S The ^{13}C-NMR spectrums of compound **3** measured in different *d*-solvents.

(1) Compound **2** measured in DMSO-d_6,100 MHz



(2) Compound **3** measured in MeCO-d_6, 100 MHz