

## SUPPLEMENTARY MATERIAL

### Two novel diterpenes from the stems and leaves of tropical seagrass

#### *Enhalus acoroides* in the South China Sea <sup>1</sup>

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**Abstract:** Two novel diterpenes Enhoidin A (1) and Enhoidin B (2) featuring an unusual gibberellane skeleton were isolated from the stems and leaves of *Enhalus acoroides*. Their structures were elucidated on the basis of spectroscopic analysis including 1D and 2D NMR techniques and HR-ESI-MS. This is the first time that this type of lactone ring between C-18 and C-20 has been found among gibberellanes from the tropical seagrasses. Evaluation of the all compounds for cytotoxicity against four human cancer cell lines (MCF-7, HCT-116, HepG-2 and HeLa), and showed moderate cytotoxic activities.

**Keywords:** tropical seagrass; *Enhalus acoroides*; diterpene; cytotoxic activity

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Figure S1.  $^1\text{H}$ -NMR (600 MHz, pyridine) spectrum of the new compound **1**

Figure S2.  $^{13}\text{C}$ -APT (150 MHz, pyridine) spectrum of the new compound **1**

Figure S3. HSQC spectrum of the new compound **1**

Figure S4. HMBC spectrum of the new compound **1**

Figure S5.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of the new compound **1**

Figure S6. NOESY spectrum of the new compound **1**

Figure S7.  $^1\text{H}$ -NMR (600 MHz, pyridine) spectrum of the new compound **2**

Figure S8.  $^{13}\text{C}$ -APT (150 MHz, pyridine) spectrum of the new compound **2**

Figure S9. HSQC spectrum of the new compound **2**

Figure S10. HMBC spectrum of the new compound **2**

Figure S11.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of the new compound **2**

Figure S12. NOESY spectrum of the new compound **2**

Figure S13. (a) Key  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC Correlations of Compound **1**; (b) Key NOE Correlations of Compound **1**

Table S1  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR (600, 150MHz) assignments for **1** and **2** (pyridine)

Table S2 In vitro cytotoxicity of compounds **1-6**

Figure S1.  $^1\text{H}$ -NMR (600 MHz, pyridine) spectrum of the new compound **1**

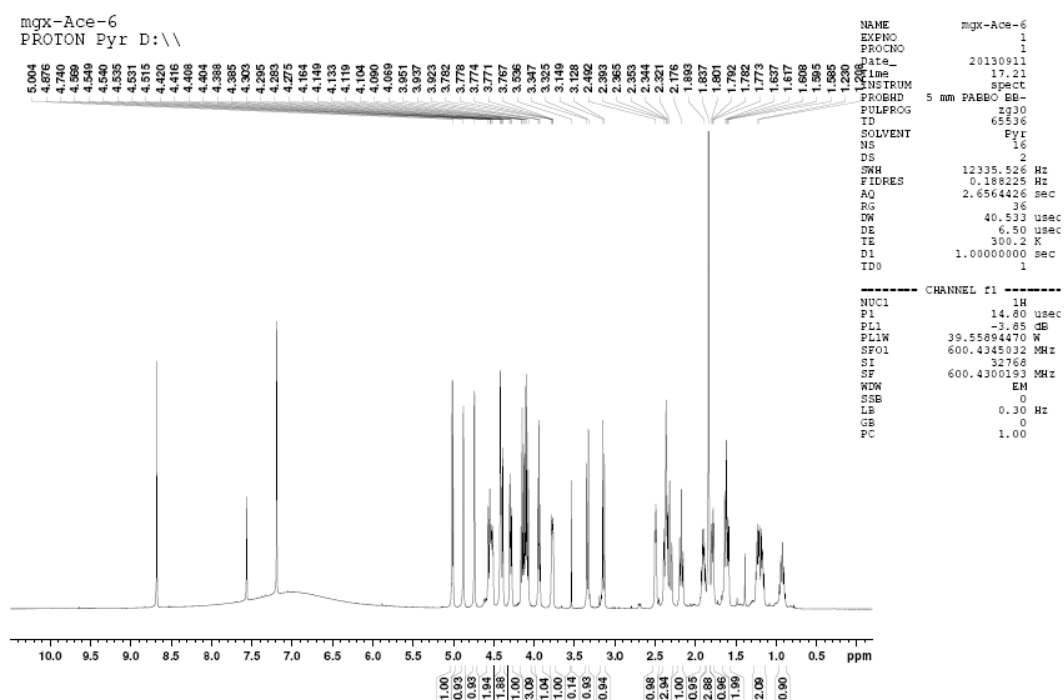


Figure S2.  $^{13}\text{C}$ -APT (150 MHz, pyridine) spectrum of the new compound **1**

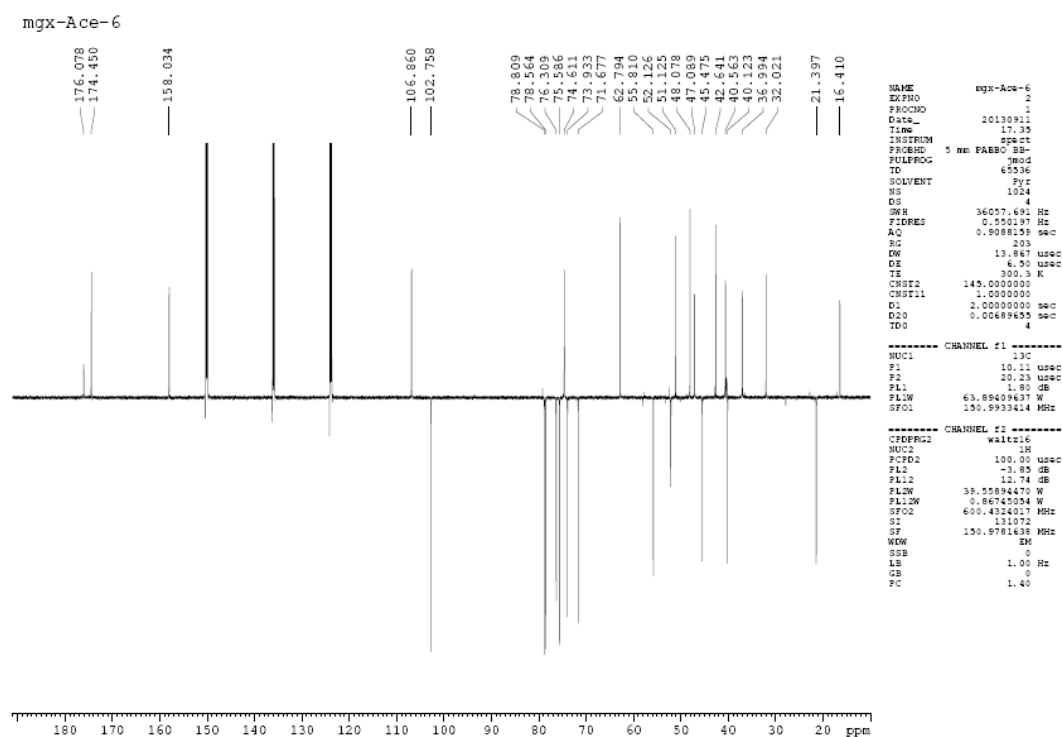


Figure S3. HSQC spectrum of the new compound **1**

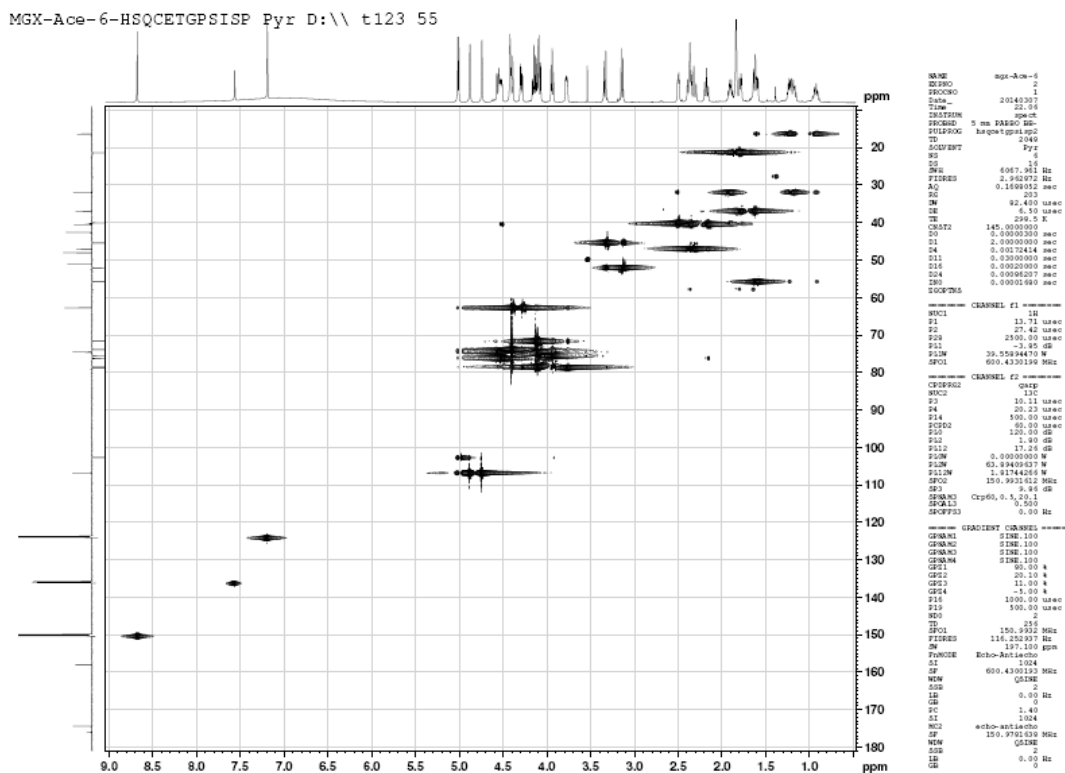


Figure S4. HMBC spectrum of the new compound **1**

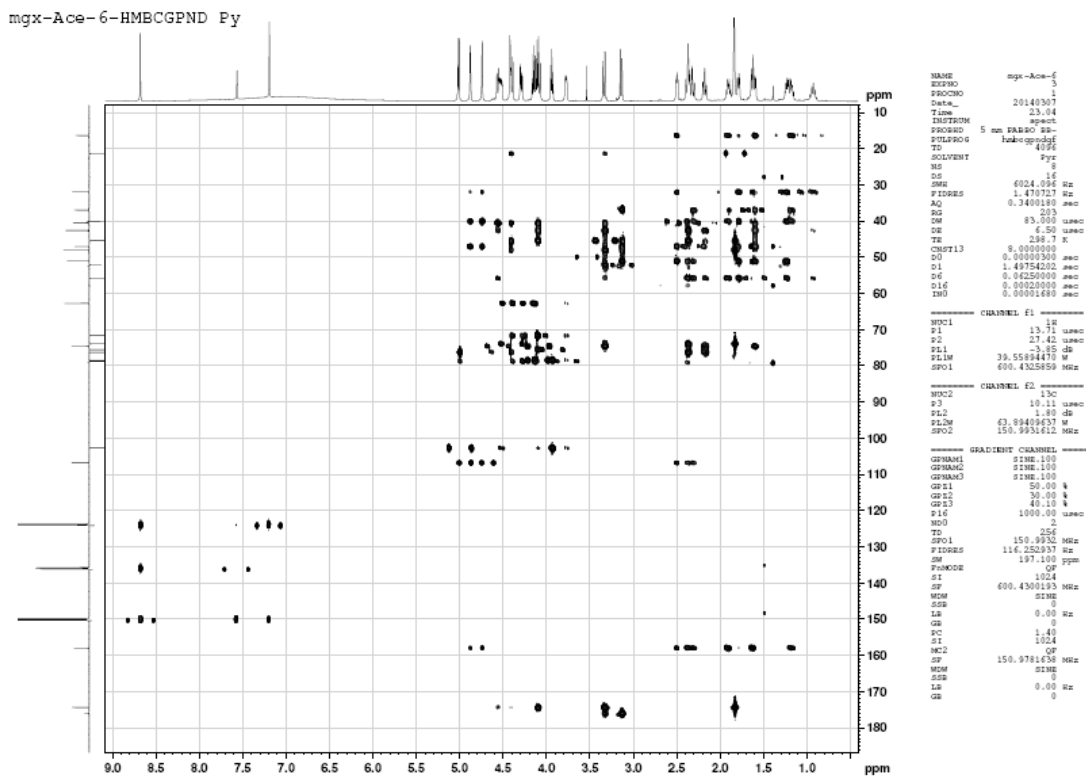


Figure S5.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of the new compound **1**

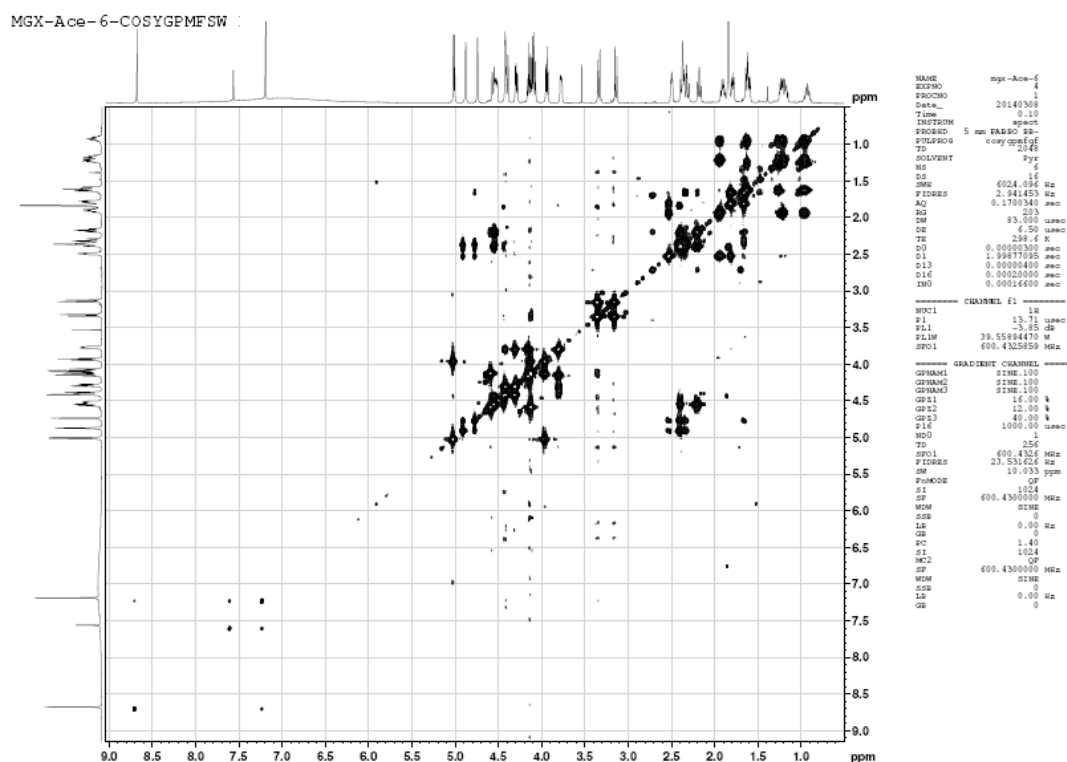


Figure S6. NOESY spectrum of the new compound **1**

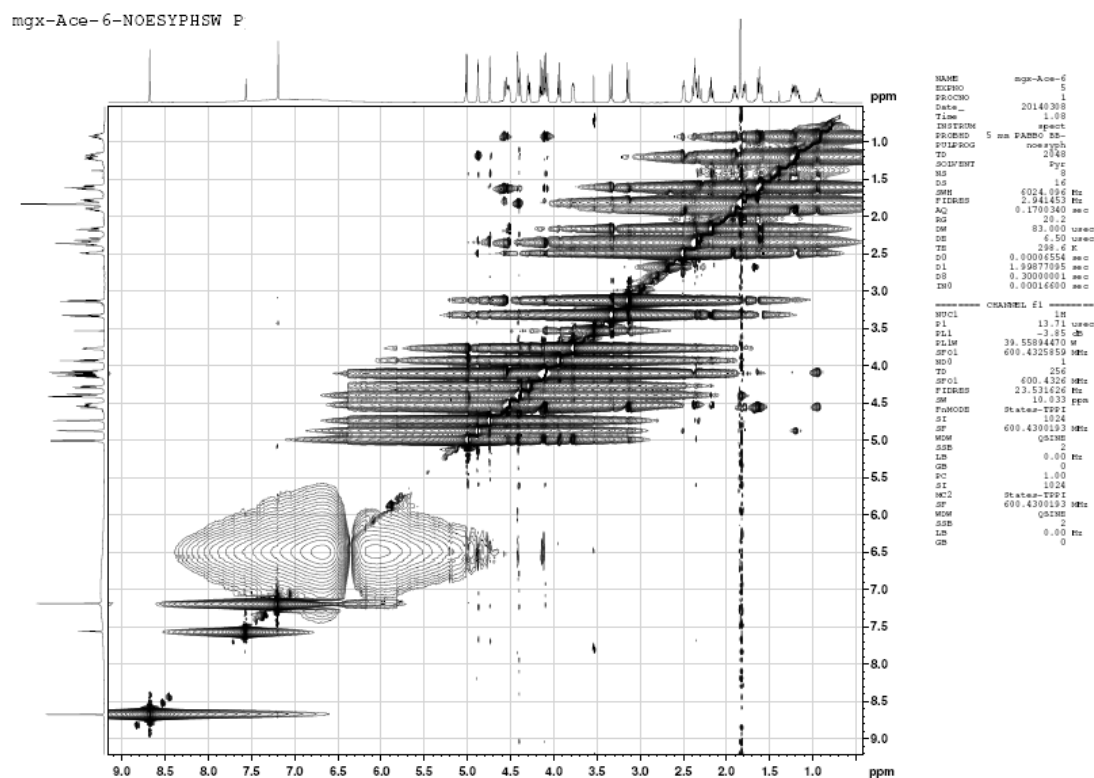


Figure S7.  $^1\text{H}$ -NMR (600 MHz, pyridine) spectrum of the new compound **2**

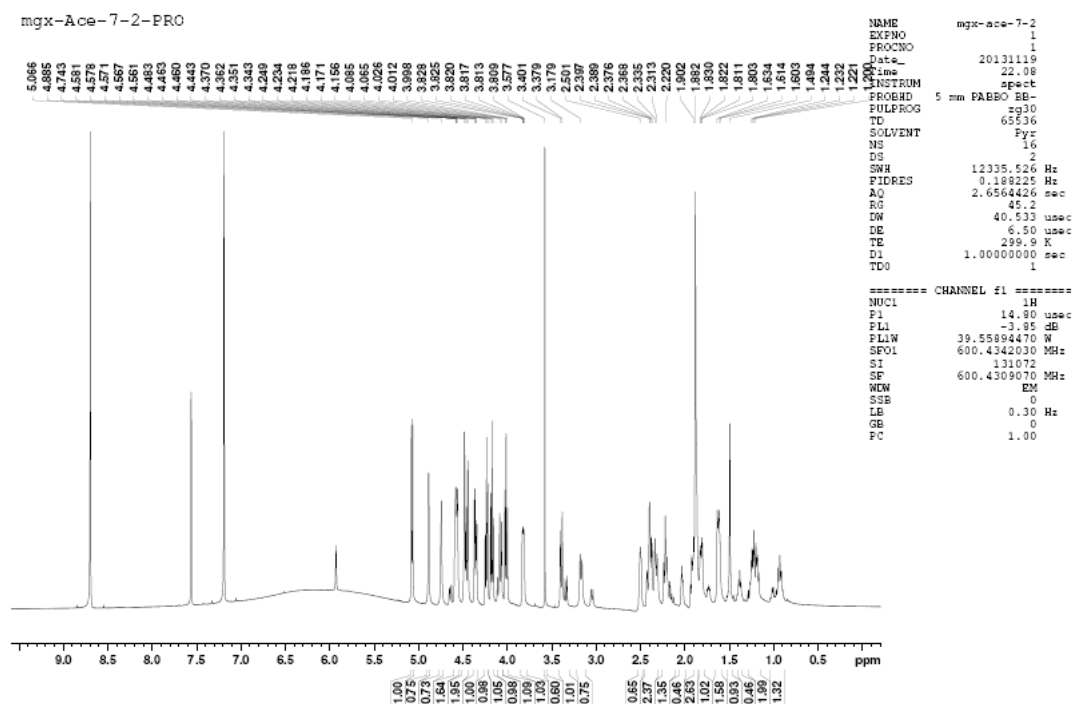
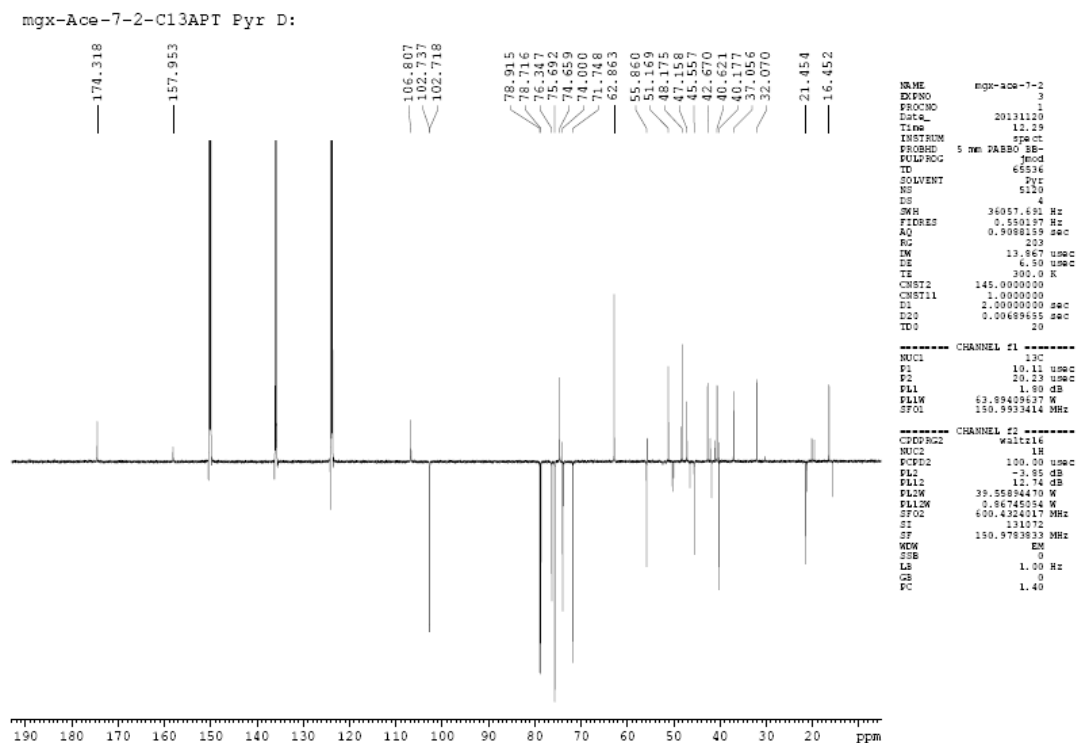
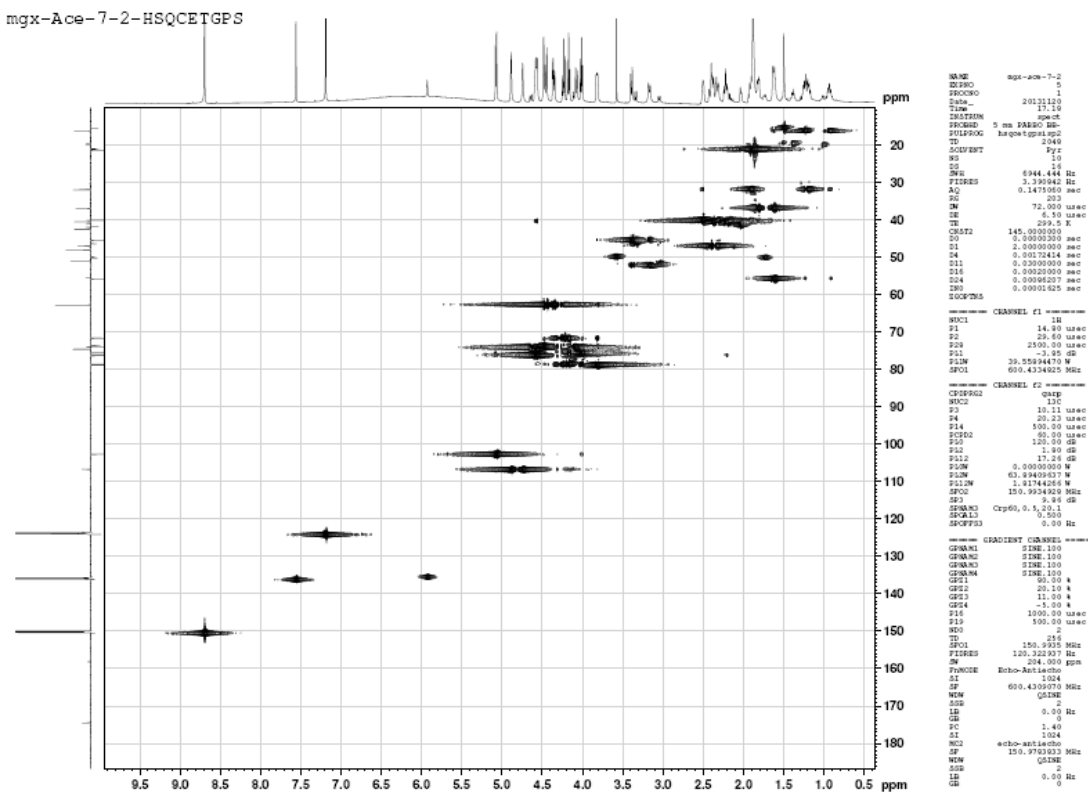


Figure S8.  $^{13}\text{C}$ -APT (150 MHz, pyridine) spectrum of the new compound **2**



max-Ace-7-2-HSQCETGPS



mgx-Ace-7-2-HMBCFPND :

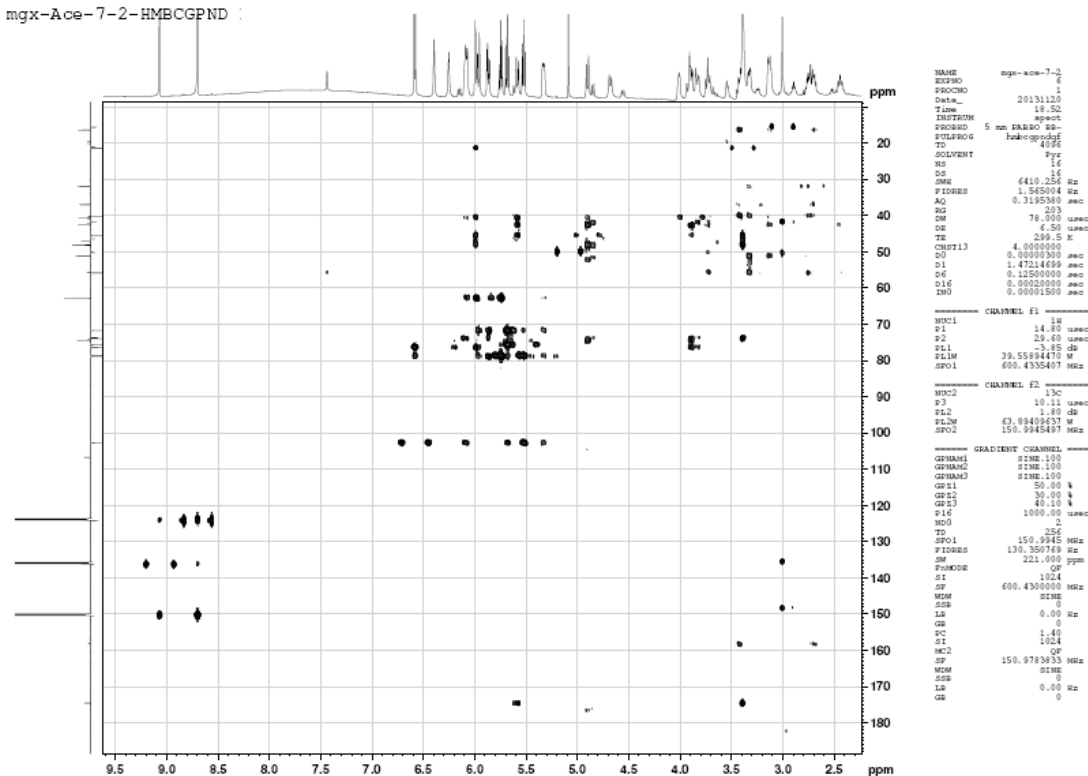


Figure S11.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of the new compound **2**

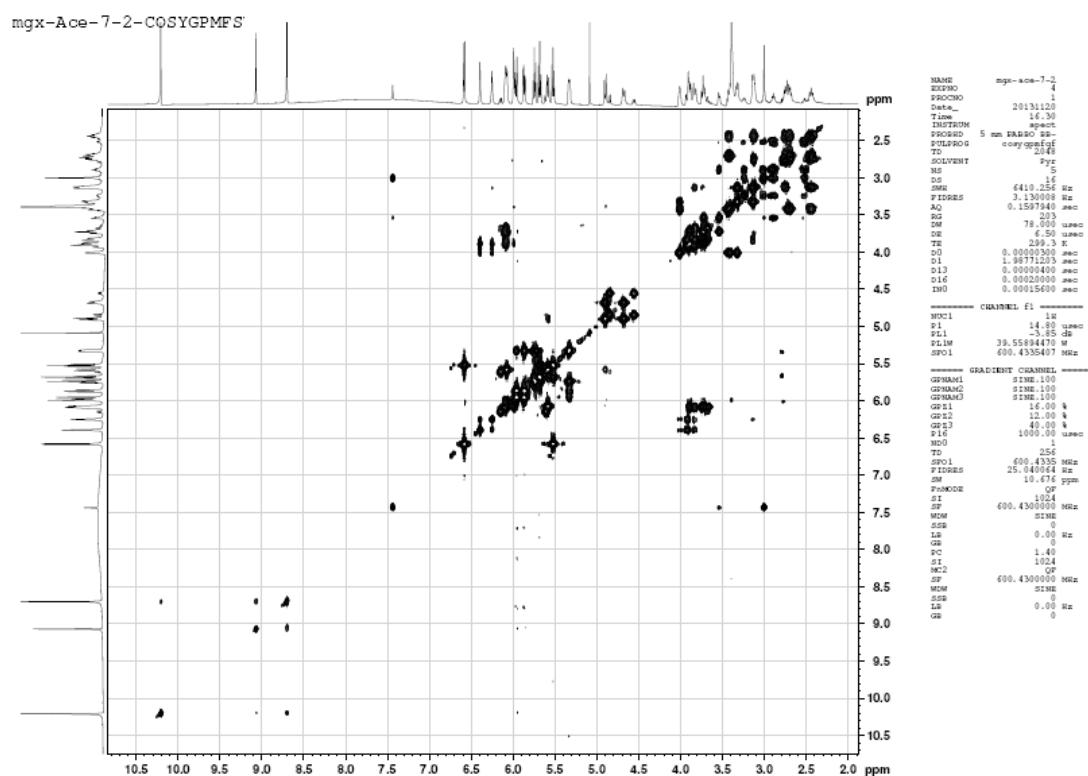


Figure S12. NOESY spectrum of the new compound **2**

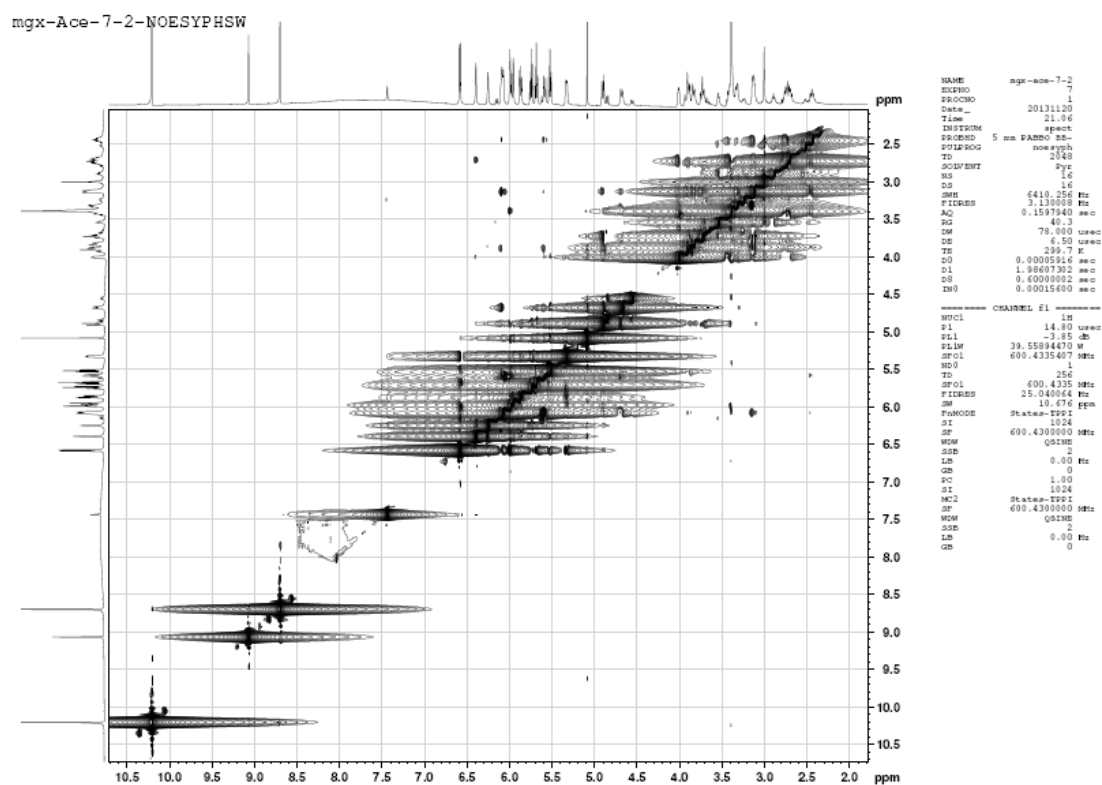




Figure S13. (a) Key  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC Correlations of Compound **1**; (b) Key NOE Correlations of Compound **1**

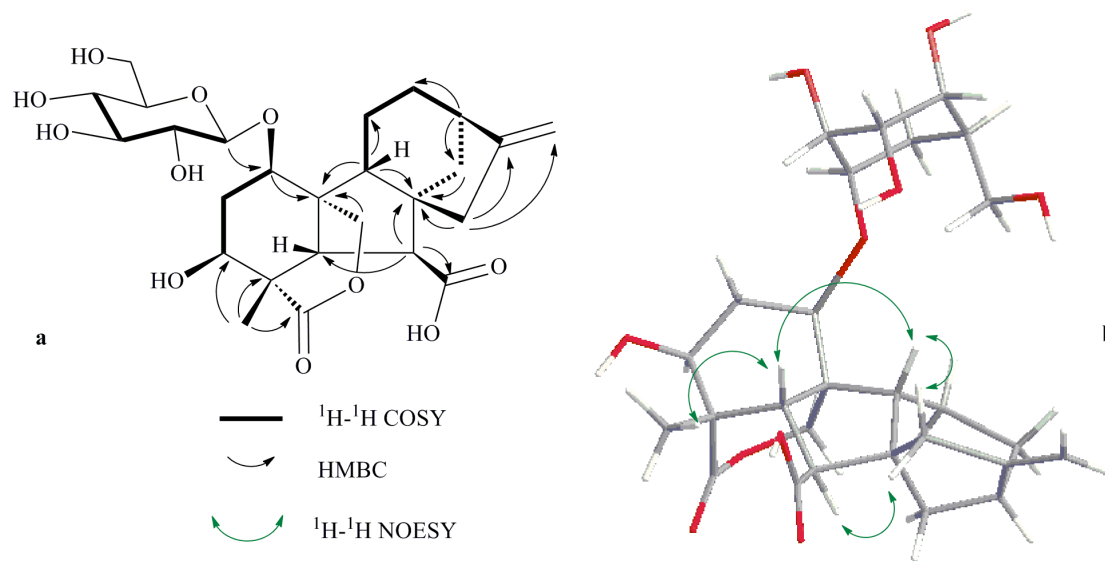


Table S1 <sup>1</sup>H-NMR and <sup>13</sup>C-NMR (Pyridine, 600, 150MHz) assignments for **1** and **2**

Position	<b>1</b>		<b>2</b>	
	$\delta_C$	$\delta_H$ (J in Hz)	$\delta_C$	$\delta_H$ (J in Hz)
1	76.3	4.53, m	76.3	4.57, m
2	40.6	2.18, m; 2.32, m	40.3	2.32, m; 2.17, m
3	73.9	4.38, dd, 12.0, 1.8	73.8	4.64, dd, 12.0, 1.2
4	48.1		48.3	
5	45.5	3.33, d, 12.6	46.5	3.34, d, 12.6
6	52.1	3.13, d, 12.6	50.3	3.05, d, 12.6
7	176.1		176.2	
8	51.1		55.7	
9	55.8	1.60, dd, 13.8, 6.0	55.9	1.60, dd, 13.8, 6.0
10	42.6		42.1	
11	16.4	0.94, m; 1.24, m	19.7	1.00, m; 1.51, m
12	32.0	1.16, m; 1.90, m	20.2	1.42, m; 1.74, m
13	40.1	2.49, m	41.8	2.03, d, 7.2
14	37.0	1.62, m; 1.79, m	41.1	2.21, m; 2.02, m
15	47.1	2.39, m; 2.32, m	135.8	5.93, s
16	158.0		149.4	
17	106.9	4.87, s; 4.74, s	15.7	1.50, s
18	174.5		174.6	
19	21.4	1.84, s	21.2	1.87, s
20	74.6	4.57, d, 12.0;	74.1	4.10, d, 12.0
		4.10, d, 12.0		4.64, d, 12.0
Glu-1	102.8	5.01, d, 7.8	102.8	5.09, d, 7.8
Glu-2	75.6	3.94, m	75.7	4.02, m
Glu-3	78.6	4.07, m	78.7	4.16, m
Glu-4	71.7	4.15, m	71.8	4.24, m
Glu-5	78.8	3.78, m	79.0	3.82, m

Glu-6	62.8	4.28,m; 4.39,m	62.9	4.47, m; 4.36, m
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Note: Assignments were accomplished using HSQC, HMBC,  $^1\text{H}$ - $^1\text{H}$  COSY and NOESY experiments.

Table S2 In vitro cytotoxicity of compounds **1-6**

Compounds	IC <sub>50</sub> (uM) <sup>a</sup>			
	MCF-7	HCT-116	HepG-2	HeLa
<b>1</b>	45.4± 1.8	57.3± 3.5	40.2± 2.4	>100
<b>2</b>	82.5± 3.9	77.3± 4.8	>100	>100
<b>3</b>	38.6± 3.2	68.7± 4.1	56.6± 2.5	45.5± 1.6
<b>4</b>	69.6± 4.9	80.2± 3.2	>100	35.8± 2.3
<b>5</b>	76.2± 4.6	88.9± 3.8	>100	>100
<b>6</b>	30.5± 1.6	28.5± 2.7	32.2± 1.8	47.5± 2.8
<b>Doxorubicin<sup>c</sup></b>	1.35± 0.07	1.14 ± 0.04	0.9± 0.02	1.21 ± 0.08

<sup>a</sup> IC<sub>50</sub> = inhibitory concentration 50%; Values are means ± SD of triplicate experiments. <sup>c</sup> Positive control sunatance