

## FULL PAPER

Three New Pregnane Alkaloids from *Pachysandra terminalis*

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Three new pregnane alkaloids, pachystermine C (**1**), pachysanamine A (**2**), and pachysanamine B (**3**), together with four known ones, pachystermine B (**4**), pachysanamine A (**5**), (20*S*)-20-(dimethylamino)-16 $\alpha$ -hydroxy-3 $\beta$ -(3'-isopropyl)lactam-5 $\alpha$ -pregnan-4-one (**6**), and *E*-salignone (**7**), were isolated from *Pachysandra terminalis*. The chemical structures of the new alkaloids were elucidated by spectroscopic methods. All the compounds were evaluated for their inhibitory activities against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines, some of the compounds showed stronger cytotoxicity for the test cell lines, especially compounds **2**, **3**, and **7**.

**Keywords:** Pregnane alkaloids, Buxaceae, *Pachysandra terminalis*, Cytotoxicity.

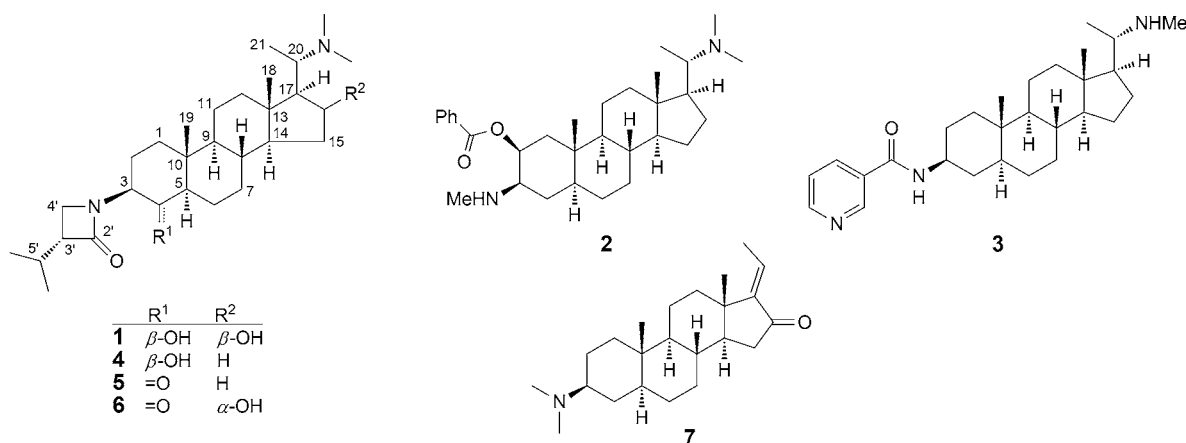
## Introduction

*Pachysandra* is a genus of evergreen perennials or subshrubs, belonging to the boxwood family Buxaceae, and *Pachysandra terminalis* (common names Japanese pachysandra), is native to Japan, Korea, and P. R. China [1][2]. A series of chemical study of *Pachysandra* genus has been carried out, which led to the isolation of many pregnane alkaloids. In particular, some of them had shown antitumor and antiulcer activities [3 – 12]. It is known that the habitat has a strong impact on the secondary metabolites of the plants. Though many phytochemical studies on the plants of *Pachysandra* genus had been carried out, there was no report on the *P. terminalis* which grows in P. R. China. And in this investigation,

three new pregnane alkaloids (**1** – **3**), together with four known ones, pachystermine B (**4**) [4][13], pachysteramine A (**5**) [4][13], (20*S*)-20-(dimethylamino)-16 $\alpha$ -hydroxy-3 $\beta$ -(3'-isopropyl)lactam-5 $\alpha$ -pregnan-4-one (**6**) [14], and *E*-salignone (**7**) [15], were isolated from *P. terminalis* growing in P. R. China (Fig. 1). Herein, the structural characterization of compounds **1** – **3** and their cytotoxicities were given.

## Results and Discussion

Pachystermine C (**1**) was obtained as a white powder, for which the molecular formula was assigned as C<sub>29</sub>H<sub>50</sub>N<sub>2</sub>O<sub>3</sub> on the basis of the HR-EI-MS (*m/z* 474.3812, [*M*]<sup>+</sup>). And the positive FAB-MS exhibited a diagnostic fragment of

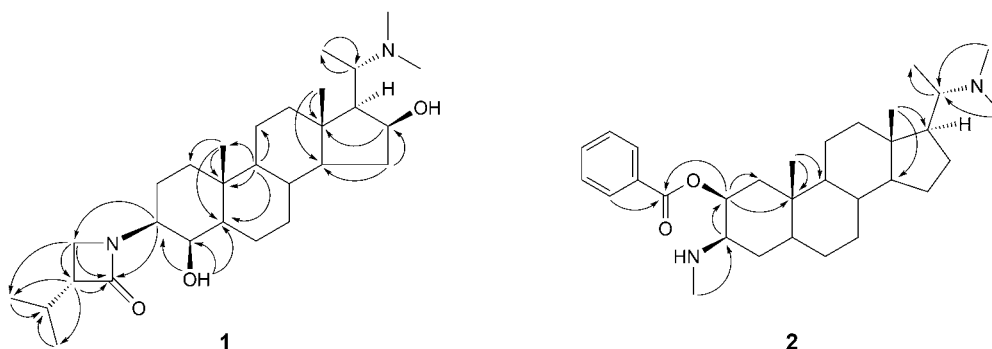
Fig. 1. Structures of compounds **1** – **7**.

*N*-ethylidene-*N*-dimethylaminium at  $m/z$  72 (100%), which suggested a 20-(dimethylamino) pregnane skeleton [16]. The  $^1\text{H}$ -NMR spectrum (Table 1) showed characteristic signals:  $\delta(\text{H})$  0.86 (3H, *s*, Me(18)), 1.07 (3H, *s*, Me(19)), 0.92 (3H, *d*,  $J = 6.5$  Hz, Me(21)), 2.23 (6H, *s*, Me<sub>2</sub>(N)). In addition,  $^{13}\text{C}$ -DEPT data (Table 1) showed signals for seven methyls, eight methylenes, eleven methines (including two oxygenated:  $\delta(\text{C})$  72.3 (*d*) and  $\delta(\text{C})$  75.5 (*d*)), and four quaternary carbons (including a carbonyl one:  $\delta(\text{C})$  170.0 (*s*)). Considering the abundance of pregnane alkaloids in the *Pachysandra* genus, compound **1** was proposed to have a basic skeleton of 20-(dimethylamino)pregnane.

A comparison of the molecular formula of **1** and **4** revealed that there was an O-atom more in **1** than **4**. The spectroscopic data of **1** and **4** were similar, and the only difference was that **1** had one more OH group. The additional OH group was positioned at C(16) due to the signals shifted downfield to  $\delta(\text{C})$  72.5 (C(16) in **1** from  $\delta(\text{C})$  27.6 C(16) in **4**, and  $\delta(\text{C})$  34.7 (C(15) in **1** from  $\delta(\text{C})$  24.0 C(15) in **4**). In the HMBC spectrum (Fig. 2), the following signal correlations were observed: H–C(16) ( $\delta(\text{H})$  4.30 (*dd*,  $J = 13.8, 7.6$ )) with C(13), H–C(15) ( $\delta(\text{H})$  2.14 – 2.20 (*m*)) with C(13), C(14), and C(16), H–C(5) ( $\delta(\text{H})$  1.08 – 1.14 (*m*)) with C(4), H–C(3) ( $\delta(\text{H})$  3.17 (*dt*,  $J = 14.0, 6.0$ )) with C(2') and C(4'), and these confirmed

Table 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of compounds **1** – **3**.  $\delta$  in ppm,  $J$  in Hz.

Position	<b>1</b>		<b>2</b>		<b>3</b>	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
1	1.71 – 1.78 ( <i>m</i> ), 0.89 – 0.99 ( <i>m</i> )	37.4 ( <i>t</i> )	1.81 – 1.87 ( <i>m</i> ), 1.54 – 1.63 ( <i>m</i> )	38.2 ( <i>t</i> )	1.68 – 1.73 ( <i>m</i> ), 0.92 – 0.99 ( <i>m</i> )	37.3 ( <i>t</i> )
2	1.75 – 1.83 ( <i>m</i> ), 1.32 – 1.36 ( <i>m</i> )	25.8 ( <i>t</i> )	5.32 ( <i>dt</i> , $J = 12.3, 4.4$ )	73.9 ( <i>d</i> )	1.77 – 1.87 ( <i>m</i> ), 1.48 – 1.57 ( <i>m</i> )	26.8 ( <i>t</i> )
3	3.17 ( <i>dt</i> , $J = 14.0, 6.0$ )	58.9 ( <i>d</i> )	3.02 – 3.08 ( <i>m</i> )	57.8 ( <i>d</i> )	4.19 – 4.25 ( <i>m</i> )	48.7 ( <i>d</i> )
4	4.04 ( <i>m</i> )	72.3 ( <i>d</i> )	1.42 – 1.51 ( <i>m</i> ), 1.14 – 1.33 ( <i>m</i> )	27.7 ( <i>t</i> )	1.49 – 1.54 ( <i>m</i> ), 1.04 – 1.07 ( <i>m</i> )	35.1 ( <i>t</i> )
5	1.08 – 1.14 ( <i>m</i> )	49.1 ( <i>d</i> )	1.54 – 1.63 ( <i>m</i> )	38.2 ( <i>d</i> )	1.04 – 1.17 ( <i>m</i> )	45.2 ( <i>d</i> )
6	1.36 – 1.43 ( <i>m</i> ), 1.23 – 1.36 ( <i>m</i> )	20.2 ( <i>t</i> )	1.54 – 1.65 ( <i>m</i> ), 1.43 – 1.54 ( <i>m</i> )	30.7 ( <i>t</i> )	1.73 – 1.79 ( <i>m</i> ), 1.16 – 1.27 ( <i>m</i> )	28.7 ( <i>t</i> )
7	1.76 – 1.83 ( <i>m</i> ), 0.82 – 0.96 ( <i>m</i> )	32.4 ( <i>t</i> )	1.64 – 1.72 ( <i>m</i> ), 0.88 – 1.02 ( <i>m</i> )	31.7 ( <i>t</i> )	1.63 – 1.68 ( <i>m</i> ), 1.46 – 1.54 ( <i>m</i> )	32.0 ( <i>t</i> )
8	1.44 – 1.55 ( <i>m</i> )	34.8 ( <i>d</i> )	1.54 – 1.63 ( <i>m</i> )	34.7 ( <i>d</i> )	1.33 – 1.42 ( <i>m</i> )	35.3 ( <i>d</i> )
9	0.55 – 0.59 ( <i>m</i> )	54.5 ( <i>d</i> )	0.83 – 0.93 ( <i>m</i> )	54.1 ( <i>d</i> )	0.65 – 0.73 ( <i>m</i> )	54.3 ( <i>d</i> )
10		35.9 ( <i>s</i> )		37.3 ( <i>s</i> )		36.0 ( <i>s</i> )
11	1.86 – 1.99 ( <i>m</i> ), 1.47 – 1.56 ( <i>m</i> )	21.5 ( <i>t</i> )	1.47 – 1.55 ( <i>m</i> ), 1.22 – 1.33 ( <i>m</i> )	20.9 ( <i>t</i> )	1.24 – 1.32 ( <i>m</i> ), 1.13 – 1.19 ( <i>m</i> )	21.6 ( <i>t</i> )
12	1.77 – 1.86 ( <i>m</i> ), 1.00 – 1.07 ( <i>m</i> )	40.2 ( <i>t</i> )	1.84 – 1.93 ( <i>m</i> ), 1.02 – 1.13 ( <i>m</i> )	39.6 ( <i>t</i> )	1.85 – 1.97 ( <i>m</i> ), 1.12 – 1.20 ( <i>m</i> )	39.3 ( <i>t</i> )
13		41.7 ( <i>s</i> )		41.6 ( <i>s</i> )		42.2 ( <i>s</i> )
14	0.81 – 0.93 ( <i>m</i> )	53.4 ( <i>d</i> )	0.98 – 1.09 ( <i>m</i> )	56.5 ( <i>d</i> )	1.01 – 1.20 ( <i>m</i> )	56.8 ( <i>d</i> )
15	2.14 – 2.20 ( <i>m</i> ), 1.18 – 1.26 ( <i>m</i> )	34.7 ( <i>t</i> )	1.53 – 1.63 ( <i>m</i> ), 1.01 – 1.11 ( <i>m</i> )	24.0 ( <i>t</i> )	1.53 – 1.63 ( <i>m</i> ), 1.11 – 1.18 ( <i>m</i> )	23.9 ( <i>t</i> )
16	4.30 ( <i>dd</i> , $J = 13.8, 7.6$ )	72.5 ( <i>d</i> )	1.78 – 1.91 ( <i>m</i> ), 1.14 – 1.33 ( <i>m</i> )	27.6 ( <i>t</i> )	1.73 – 1.79 ( <i>m</i> ), 1.19 – 1.28 ( <i>m</i> )	28.4 ( <i>t</i> )
17	1.19 – 1.25 ( <i>m</i> )	55.4 ( <i>d</i> )	1.32 – 1.43 ( <i>m</i> )	54.7 ( <i>d</i> )	1.39 – 1.48 ( <i>m</i> )	56.5 ( <i>d</i> )
18	0.86 ( <i>s</i> )	14.7 ( <i>q</i> )	0.64 ( <i>s</i> )	12.3 ( <i>q</i> )	0.75 ( <i>s</i> )	12.2 ( <i>q</i> )
19	1.07 ( <i>s</i> )	14.3 ( <i>q</i> )	0.93 ( <i>s</i> )	12.7 ( <i>q</i> )	0.78 ( <i>s</i> )	12.3 ( <i>q</i> )
20	2.89 – 2.96 ( <i>m</i> )	59.7 ( <i>d</i> )	2.35 – 2.42 ( <i>m</i> )	61.1 ( <i>d</i> )	2.49 – 2.55 ( <i>m</i> )	59.0 ( <i>d</i> )
21	0.92 ( <i>d</i> , $J = 6.5$ )	9.8 ( <i>q</i> )	0.85 ( <i>d</i> , $J = 7.5$ )	9.7 ( <i>q</i> )	1.27 ( <i>d</i> , $J = 6.7$ )	19.4 ( <i>q</i> )
Me <sup>1</sup>	2.23 ( <i>s</i> )	40.2 ( <i>q</i> )	2.16 ( <i>s</i> )	39.8 ( <i>q</i> )	2.41 ( <i>s</i> )	33.0 ( <i>q</i> )
Me <sup>2</sup>			2.41 ( <i>s</i> )	34.8 ( <i>q</i> )		
1'				165.7 ( <i>s</i> )		164.4 ( <i>s</i> )
2'		170.0 ( <i>s</i> )		130.5 ( <i>s</i> )		130.8 ( <i>s</i> )
3'	2.89 – 2.95 ( <i>m</i> )	56.7 ( <i>d</i> )	8.03 ( <i>dd</i> , $J = 7.4, 1.1$ )	129.5 ( <i>d</i> )	8.90 ( <i>s</i> )	147.5 ( <i>d</i> )
4'	3.40 ( <i>t</i> , $J = 8.8$ ), 2.89 – 2.95 ( <i>m</i> )	42.6 ( <i>t</i> )	7.45 ( <i>t</i> , $J = 7.4$ )	128.4 ( <i>d</i> )		
5'	1.92 – 1.97 ( <i>m</i> )	28.0 ( <i>d</i> )	7.57 ( <i>t</i> , $J = 7.4$ )	132.9 ( <i>d</i> )	8.70 ( <i>d</i> , $J = 4.6$ )	152.0 ( <i>d</i> )
Me <sup>3</sup>	0.95 ( <i>d</i> , $J = 6.7$ ) 1.05 ( <i>d</i> , $J = 6.7$ )	19.9 ( <i>q</i> ) 19.8 ( <i>q</i> )				
6'					7.39 ( <i>dd</i> , $J = 7.5, 4.6$ )	123.5 ( <i>d</i> )
7'					8.08 ( <i>d</i> , $J = 7.5$ )	135.1 ( <i>d</i> )
OH	3.71 ( <i>d</i> , $J = 3.3$ , HO–C(4))					
NH					6.02 ( <i>d</i> , $J = 8.0$ , HN–C(3))	

Fig. 2. Key HMBCs (H → C) of **1** and **2**.

the above assignment. The HMBC data of  $\delta(\text{H})$  3.71 ( $d$ ,  $J = 3.3$ ) with C(3), C(4), and C(5) were observed, which proved that the  $\delta(\text{H})$  3.71 ( $d$ ,  $J = 3.3$ ) was the  $^1\text{H}$ -NMR signal of the HO group at C(4). The ROESY correlations of  $\text{H}\alpha\text{-C}(16)$  with  $\text{H}\alpha\text{-C}(17)$ ,  $\text{H}\alpha\text{-C}(15)$ , and  $\text{H}\alpha\text{-C}(3)$  with  $\text{H}\alpha\text{-C}(4)$ ,  $\text{H}\alpha\text{-C}(5)$ , suggested that the substituents at C(3), C(4), and C(16) all had  $\beta$ -orientations. Therefore, compound **1** was elucidated as (20*S*)-20-(dimethylamino)-4 $\beta$ ,16 $\beta$ -dihydroxy-3 $\beta$ -(3'- $\alpha$ -isopropyl)lactam-5 $\alpha$ -pregnane.

Pachysanamine A (**2**) was isolated as white powder. The molecular formula was determined to be  $\text{C}_{31}\text{H}_{48}\text{N}_2\text{O}_2$  by HR-EI-MS ( $m/z$  480.3715,  $[\text{M}]^+$ ). And the positive FAB-MS also exhibited a diagnostic fragment at  $m/z$  72 (100%), which suggested a 20-(dimethylamino)pregnane [16]. The  $^1\text{H}$ -NMR spectra (Table 1) displayed the presence of six Me signals:  $\delta(\text{H})$  0.64 (3 H,  $s$ , Me-C(18)), 0.93 (3 H,  $s$ , Me-C(19)), 0.85 (3 H,  $d$ ,  $J = 7.5$  Hz, Me-C(21)), 2.41 (3 H,  $s$ , Me(N)-C(3)), 2.23 (6 H,  $s$ , Me<sub>2</sub>(N)-C(20)).

Careful comparison of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of **2** (Table 1) and pachysamine J [17] revealed that the two compounds have the similar skeleton except for the substituent group at N-C(3) and HO-C(2). The senecioid group at N-C(3) in pachysamine J was replaced by a methyl group, while the H-atom of HO-C(2) was replaced by a benzoyl group, which was confirmed by the HMBC experiments (Fig. 2). In the HMBC spectrum, the long-range correlations were observed from H-C(2) ( $\delta(\text{H})$  5.32 ( $dt$ ,  $J = 12.3, 4.4$  Hz)) to C(1), C(10) and C(1'), from H-C(1) ( $\delta(\text{H})$  1.81 – 1.87 ( $m$ ), 1.54 – 1.63 ( $m$ )) to C(2), C(10), and from Me-N(C(3)) ( $\delta(\text{H})$  2.41 ( $s$ )) to C(3). The relative configurations of HO-C(2) and C(3) were assigned as  $\beta$ -orientation by correlations of  $\text{H}\alpha\text{-C}(2)$  with  $\text{H}\alpha\text{-C}(3)$ , and  $\text{H}\alpha\text{-C}(3)$  with  $\text{H}\alpha\text{-C}(2)$ ,  $\text{H}\alpha\text{-C}(5)$ . So, compound **2** was characterized as (20*S*)-(dimethylamino)-3 $\beta$ -*N*-methylamino-2 $\beta$ -benzoyloxy-5 $\alpha$ -pregnane.

Pachysanamine B (**3**) was obtained as white powder. Its molecular formula  $\text{C}_{28}\text{H}_{43}\text{N}_3\text{O}$ , determined from the HR-EI-MS, had a  $\text{CH}_2$ -group less than that of *epi*-pachysamine B [4]. The  $^1\text{H}$ -NMR spectrum of **3** (Table 1) showed four Me signals:  $\delta(\text{H})$ : 0.75 (3 H,  $s$ , Me-C(18)), 0.78 (3 H,  $s$ , Me-C(19)), 1.27 (3 H,  $d$ ,  $J = 6.7$  Hz, Me-C(21)), 2.41 (3 H,  $s$ , Me(N)-C(20)), which were

characteristic signals of a pregnane skeleton. Analysis of the  $^{13}\text{C}$ -NMR spectrum (Table 1) indicated the presence of pyridine ring:  $\delta(\text{C})$ : 130.8 ( $s$ , C(2')), 152.0 ( $d$ , C(3')), 147.5 ( $d$ , C(5')), 123.5 ( $d$ , C(6')), 135.1 ( $d$ , C(7')). And there were no palpable differences in the NMR spectrum between **3** and *epi*-pachysamine B, except for a Me group less at N-C(20) in **3** than *epi*-pachysamine B. Moreover, the HMBC correlations of **3** (Fig. 3) were observed from H-C(2) ( $\delta(\text{H})$  1.77 – 1.87 ( $m$ ), 1.48 – 1.57 ( $m$ )) to C(3), from H-C(3) ( $\delta(\text{H})$  4.19 – 4.25 ( $m$ )) to C(1'). The HMBC correlations of  $\delta(\text{H})$  6.02 ( $d$ ,  $J = 8.0$ ) with C(3) and C(3) were observed, which showed that the  $\delta(\text{H})$  6.02 ( $d$ ,  $J = 8.0$ ) was the  $^1\text{H}$ -NMR signal of amide NH proton at C(3). Consequently, the structure of **3** was elucidated as (20*S*)-(methylamino)-3 $\beta$ -pyridinecarbonylamino-5 $\alpha$ -pregnane.

Compounds **1** – **7** (purity > 90%) were tested for their cytotoxic activities *in vitro* against HL-60, SMMC7721, A549, MCF-7, and SW-480 cell lines (Table 2), using the improved MTT method as previously described [17]. Compared with positive control cisplatin (DDP; Sigma, St. Louis, USA, purity > 98%), compound **3** has obvious cytotoxicity against all the cell lines with the  $\text{IC}_{50}$  value of  $2.4 \pm 0.3$ ,  $7.3 \pm 0.8$ ,  $3.6 \pm 0.3$ ,  $3.1 \pm 0.3$ , and  $3.7 \pm 0.4$   $\mu\text{M}$ , respectively. Compound **2** showed moderate cytotoxicity against all the cell lines with the  $\text{IC}_{50}$  value of  $3.8 \pm 0.5$ ,  $15.7 \pm 1.4$ ,  $10.7 \pm 0.5$ ,  $13.9 \pm 0.8$ , and  $11.4 \pm 0.6$   $\mu\text{M}$ , respectively. Compound **7** showed selective cytotoxicity against A-549, and MCF-7 cell lines with the

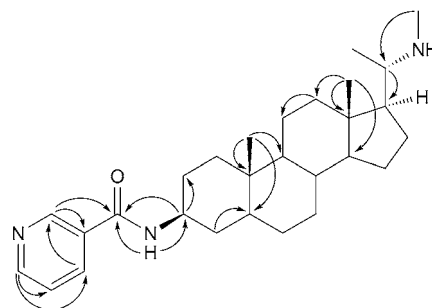
Fig. 3. Key HMBCs (H → C) of **3**.

Table 2. Cytotoxicity of compounds **1**–**7** toward different cancer cells<sup>a)</sup>

Compound	HL-60	SMMC-7721	A-549	MCF-7	SW480
<b>1</b>	18.8 ± 1.8	28.1 ± 2.2	15.7 ± 0.8	15.3 ± 0.8	14.0 ± 1.2
<b>2</b>	3.8 ± 0.5	15.7 ± 1.4	10.7 ± 0.5	13.9 ± 0.8	11.4 ± 0.6
<b>3</b>	2.4 ± 0.3	7.3 ± 0.7	3.6 ± 0.3	3.1 ± 0.3	3.7 ± 0.4
<b>4</b>	16.0 ± 0.8	36.9 ± 3.3	17.1 ± 1.3	17.4 ± 1.1	17.2 ± 1.2
<b>5</b>	14.3 ± 0.7	35.2 ± 2.9	19.1 ± 1.4	15.9 ± 1.3	16.6 ± 1.0
<b>6</b>	14.3 ± 0.5	24.3 ± 2.1	17.1 ± 1.3	15.7 ± 1.2	12.9 ± 1.0
<b>7</b>	5.5 ± 0.3	15.2 ± 0.6	6.3 ± 0.7	4.1 ± 0.4	9.4 ± 1.0
Cisplatin	1.0 ± 0.2	14.8 ± 0.7	13.6 ± 0.9	17.1 ± 1.3	15.6 ± 1.5

<sup>a)</sup> Results are expressed as  $IC_{50}$  values in  $\mu\text{M}$  (mean ± SD,  $n = 3$ ).

$IC_{50}$  value of  $6.3 \pm 0.7$ ,  $4.1 \pm 0.4$ . The other compounds showed low inhibitory activity against the tumor cells.

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## Experimental Part

### General

Solvents used for extraction and isolation were distilled prior to use. TLC: precoated silica gel *GF<sub>245</sub>* glass plates (*Qingdao Marine Chemical Inc.*, Qingdao, P. R. China). Column chromatography (CC): silica gel (200–300 mesh, *Qingdao Marine Chemical Inc.*), alumina (*Jinshan Works*, Shanghai, P. R. China), and *Sephadex LH-20* (*Pharmacia*, Uppsala, Sweden). Optical rotations: *Horiba SEPA-300* polarimeter. IR Spectra: *Bio-Rad FTS-135* infrared spectrophotometer (Berkeley, USA);  $\tilde{\nu}$  in  $\text{cm}^{-1}$ . 1D- and 2D-NMR spectra: *Bruker AV-400*, *DRX-500*, and/or *AV-600* instruments (Billerica, USA) in  $\text{CDCl}_3$ ;  $\delta$  in ppm rel. to the solvent signals,  $J$  in Hz. MS: *Autospec Premier P776* mass spectrometer (Washington, D.C., USA; the used matrix material was glycerol); in  $m/z$  (rel. %).

### Plant Material

The whole plants of *P. terminalis* were collected at Nanjing City, Jiangsu Province of P. R. China, in March 2009. The plant material was identified by Prof. *Xi-Wen Li* and a voucher (No. KIB 20090503d) has been deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

### Extraction and Isolation

Air-dried roots of *P. terminalis* (2 kg) were extracted three times with MeOH. After removal of the solvent under reduced pressure, the residue was obtained. This residue was dissolved in  $\text{H}_2\text{O}$  and adjusted to pH 2 with 3% HCl. The acid-soluble fraction was alkalized to pH 9 with 5% NaOH followed by exhaustive extraction ( $5 \times$ ) with  $\text{CHCl}_3$ .  $\text{CHCl}_3$ -soluble material (50 g) was roughly separated by CC on  $\text{SiO}_2$  ( $\text{CHCl}_3/\text{MeOH}$  1:0 → 0:1) to give

four fractions, *Frs. A1*–*A4*. *Fr. A1* was chromatographed over an alumina column with a mixture of petroleum ether (PE)/acetone (1:0 → 4:1) and a silica gel column with a mixture of PE/acetone/ $\text{Et}_2\text{NH}$  (80:2:1 → 20:2:1) followed by *Sephadex LH-20* CC eluted with MeOH to afford **1** (58 mg) and **4** (12 mg). *Fr. A2* eluted with PE/acetone/ $\text{Et}_2\text{NH}$  (100:5:1 → 100:40:10) was separated by CC on  $\text{SiO}_2$  and *LH-20* eluted with MeOH to yield **2** (5 mg) and **5** (120 mg). *Fr. A3* was chromatographed over the alumina column with PE/acetone (100:10 → 100:60) and  $\text{SiO}_2$  with PE/acetone/ $\text{Et}_2\text{NH}$  (100:20:4 → 100:60:15) to yield **6** (14 mg). *Fr. A4* was also chromatographed over the alumina column with PE/acetone/ $\text{Et}_2\text{NH}$  (100:20:4 → 100:60:10) and  $\text{SiO}_2$  with PE/acetone/ $\text{Et}_2\text{NH}$  (100:20:4 → 100:60:15) to yield **3** (37 mg) and **7** (45 mg).

**Pachystermine C** (= **(3R)-1-[(3 $\beta$ ,4 $\beta$ ,5 $\alpha$ , 16 $\beta$ ,20S)-20-(Dimethylamino)-4,16-dihydroxypregnan-3-yl]-3-(1-methylethyl)-azetidin-2-one**; **1**). White powder ( $\text{CHCl}_3$ ).  $[\alpha]_{\text{D}}^{20} = -37.2$  ( $c = 0.75$ , MeOH). IR (KBr): 3216, 2933, 1708, 1508.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: see Table 1. FAB-MS (pos.): 475 (62,  $[M + \text{H}]^+$ ), 72 (100). HR-EI-MS: 474.3812 ( $M^+$ ,  $\text{C}_{29}\text{H}_{50}\text{N}_2\text{O}_3^+$ ; calc. 474.3821).

**Pachysanamine A** (= **(2 $\beta$ ,3 $\beta$ ,5 $\alpha$ ,20S)-20-(Dimethylamino)-3-(methylamino)pregnan-2-yl Benzoate**; **2**). White powder ( $\text{CHCl}_3$ ).  $[\alpha]_{\text{D}}^{20} = -81.7$  ( $c = 0.55$ , MeOH). IR (KBr): 3456, 2967, 1711, 1506.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: see Table 1. FAB-MS (pos.): 481 (88,  $[M + \text{H}]^+$ ), 359 (10), 314 (9), 72 (100). HR-EI-MS: 480.3715 ( $M^+$ ,  $\text{C}_{31}\text{H}_{48}\text{N}_2\text{O}_2^+$ ; calc. 480.3716).

**Pachysanamine B** (= **N-[(3 $\beta$ ,5 $\alpha$ ,20S)-20-(Methylamino)pregnan-3-yl]pyridine-3-carboxamide**; **3**). White powder ( $\text{CHCl}_3$ ).  $[\alpha]_{\text{D}}^{20} = -24.7$  ( $c = 1.07$ , MeOH). IR (KBr): 3305, 2928, 1656, 1545, 1458.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: see Table 1. FAB-MS (pos.): 481 (88,  $[M + \text{H}]^+$ ), 359 (10), 314 (9), 72 (100). HR-EI-MS: 437.3414 ( $M^+$ ,  $\text{C}_{28}\text{H}_{43}\text{N}_3\text{O}^+$ ; calc. 437.3406).

### Cytotoxicity Tests

The cytotoxic activity of the compounds against suspended tumor cells was determined by the MTT method. All the cells were cultured in RPMI-1640 or DMEM medium (*Hyclone*, Logan, USA), supplemented with 10% fetal bovine serum (*Hyclone*) at 37 °C in a humidified atmosphere with 5%  $\text{CO}_2$ .

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