

## FULL PAPER

Vinmajorines C–E, Monoterpenoid Indole Alkaloids from *Vinca major*by Zhi-Jun Zhang<sup>a)</sup>), Ru-Nan Du<sup>b)</sup>), Juan He<sup>b)</sup>), Xing-De Wu<sup>b)</sup>), Yan Li<sup>b)</sup>), Rong-Tao Li<sup>\*a)</sup>), and Qin-Shi Zhao<sup>\*b)</sup><sup>a)</sup> Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, P. R. China (phone: +86-871-65920569; fax: +86-871-65920570; e-mail: rongtaolikm@163.com)<sup>b)</sup> State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences (CAS), Kunming 650204, P. R. China (phone: +86-871-65223058; fax: +86-871-65215783; e-mail: qinshizhao@mail.kib.ac.cn)

Three new monoterpene indole alkaloids, vinmajorines C–E (**1–3**), along with 18 known analogues (**4–21**), were isolated from the whole plants of *Vinca major*. The new structures were elucidated as (5 $\alpha$ ,15 $\beta$ ,16R,17 $\alpha$ ,19 $\beta$ ,20 $\alpha$ ,21 $\beta$ )-10,17-dimethoxy-21-methyl-18-oxa-5,16-cycloyohimban-19-ol (**1**), (5 $\alpha$ ,15 $\beta$ ,16R,17 $\alpha$ ,20 $\alpha$ ,21 $\beta$ )-10-methoxy-21-methyl-18-oxa-5,16-cycloyohimban-17-ol (**2**), and (5 $\alpha$ ,15 $\beta$ ,16R,17 $\alpha$ ,20 $\alpha$ ,21 $\beta$ )-10-methoxy-21-methyl-18-oxa-5,16-cycloyohimban-17-yl acetate (**3**), respectively, by extensive NMR and MS analysis and comparison with known compounds. Compounds **1–3** were evaluated for their cytotoxic activities against five human cancer cell lines, compounds **1** and **3** showing moderate cytotoxic activities.

**Introduction.** – *Vinca major* is widely distributed in Europe, Northwest Africa, and Southwest Asia. In China, this plant has been cultivated widely as an outside ornament. *V. major* is rich in monoterpene indole alkaloids possessing complex frameworks and diverse bioactivities (especially anticancer activity), which have been attracting great interest from phytochemical, biogenetic, synthetic, and biological points of view [1–11]. Many of them, such as reserpine [12], vincristine [13], and yohimbine [14], are well-known for their pharmacological significance.

In our previous article, we have reported the isolation of two new monoterpene indole alkaloids, vincamajorines A and B, which represented two new C-atom skeletons [15]. As a continuation of our search for more effective components from this plant, three new monoterpene indole alkaloids, vinmajorines C–E (**1–3**; Fig. 1), together with 18 known ones, reserpine (**4**) [16], ajmalicine (**5**) [17], 11-hydroxyajmalicine (**6**) [18], vallesiachotamine (**7**) [19], strictosamide (**8**) [20], majdine (**9**) [21], (+)-vincadifformine (**10**) [22], (–)-quebrachamine (**11**) [23], (–)-

rhaznilam (**12**) [24], tetrahydromeloscin (**13**) [25], pseudoakuammigine (**14**) [21], akuammine (**15**) [21], 10-hydroxy desacetyl-akuammiline (**16**) [26], sitsirikine (**17**) [27], isositsirikine (**18**) [28], dihydrositsirikine (**19**) [29], rhazimine (**20**) [30], and vincamine (**21**) [31], were isolated. In addition, compounds **1–3** were evaluated for their cytotoxic activities against five human cancer cell lines. Herein, we mainly describe the isolation, elucidation, and biological evaluation of compounds **1–3**.

**Results and Discussion.** – Compound **1** was obtained as colorless oil. The molecular formula C<sub>21</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub> was determined by the molecular ion peak at *m/z* 370.1899 (*M*<sup>+</sup>) in the HR-EI-MS. The IR absorption bands at 3418 cm<sup>–1</sup> suggested the presence of an OH group. The UV absorption band at 278 and 225 nm indicated a substituted indole chromophore [32], which was further supported by the <sup>1</sup>H- and <sup>13</sup>C-NMR spectra ( $\delta$ (C) 140.1 (*s*, C(2)), 104.4 (*s*, C(7)), 133.4 (*s*, C(8)), 110.2 (*d*, C(9)), 155.2 (*s*, C(10)), 111.9 (*d*, C(11)), 112.6 (*d*, C(12)) and 129.1 (*s*, C(13));  $\delta$ (H) 6.91 (*d*, *J* = 2.4, H–C(9)), 6.72 (*dd*, *J* = 7.3, 2.4, H–C(11)), and 7.18 (*d*, *J* = 7.3, H–C(12))) (Table 1). Additionally, two MeO groups ( $\delta$ (H) 3.82 (*s*) and 3.49 (*s*);  $\delta$ (C) 56.1 (*q*) and 55.4 (*q*)) were also observed in the 1D-NMR spectra. Except for the signals belonging to the indole ring and two MeO groups, the <sup>13</sup>C-NMR spectrum exhibited eleven C-atoms, including eight CH groups (two special ones at  $\delta$ (C) 93.8 and 100.9 attributed to two hemiacetal groups), two CH<sub>2</sub> groups ( $\delta$ (C) 29.2 and 33.3) and one Me group ( $\delta$ (C) 14.0). Careful comparison of the NMR data of **1** with those of alstoyunine B [33] suggested that they possessed the same C-atom skeleton. The fragments of H–C(3)/CH<sub>2</sub>(14)/H–C(15)/H–C(16), Me(18)/H–C(19)/H–C(20), CH<sub>2</sub>(6)/H–C(5)/H–C(16)/H–C(17), and H–C(15)/H–C(20)/

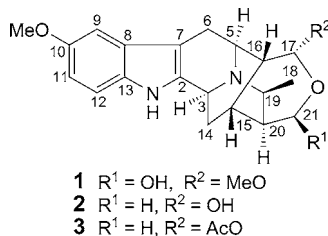
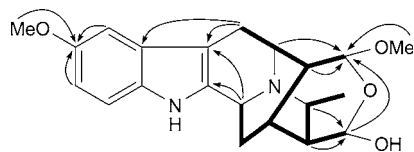
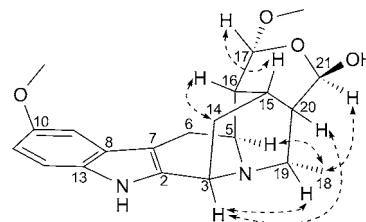
Fig. 1. The structures of compounds **1–3**<sup>1)</sup> These authors contributed equally.

Table 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR (500 and 125 MHz, resp.) Data of **1–3**.  $\delta$  in ppm,  $J$  in Hz.

Position	<b>1</b>		<b>2</b>		<b>3</b>	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
2		140.1		139.9		139.9
3	4.10 ( <i>d</i> , $J=10.4$ )	52.8	3.99 ( <i>d</i> , $J=10.1$ )	53.6	4.07 ( <i>d</i> , $J=10.5$ )	53.6
5	3.75–3.81 ( <i>m</i> )	45.0	2.98–3.03 ( <i>m</i> )	49.4	1.57–1.62 ( <i>m</i> )	49.4
6	2.89–2.96 ( <i>m</i> ), 2.58 ( <i>d</i> , $J=15.4$ )	29.2	2.91–2.95 ( <i>m</i> ), 2.86 ( <i>d</i> , $J=15.4$ )	28.2	2.94–2.95 ( <i>m</i> ), 2.96–3.00 ( <i>m</i> )	28.2
7		104.4		105.0		105.0
8		133.4		129.2		129.3
9	6.91 ( <i>d</i> , $J=2.4$ )	110.2	6.89 ( <i>d</i> , $J=2.2$ )	101.2	6.92 ( <i>d</i> , $J=2.4$ )	101.2
10		155.2		154.9		155.1
11	6.72 ( <i>dd</i> , $J=7.3, 2.4$ )	111.9	6.70 ( <i>dd</i> , $J=8.8, 2.2$ )	111.7	6.72 ( <i>dd</i> , $J=8.7, 2.4$ )	111.9
12	7.18 ( <i>d</i> , $J=7.3$ )	112.6	7.16 ( <i>d</i> , $J=8.8$ )	112.6	7.18 ( <i>d</i> , $J=8.7$ )	112.6
13		129.1		133.3		133.4
14	1.97–2.03 ( <i>m</i> ), 1.57–1.63 ( <i>m</i> )	33.3	2.11–2.17 ( <i>m</i> ), 1.32 ( <i>d</i> , $J=13.2$ )	29.9	2.19–2.22 ( <i>m</i> ), 1.38 ( <i>d</i> , $J=13.4$ )	29.9
15	2.21–2.27 ( <i>m</i> )	25.3	2.05–2.09 ( <i>m</i> )	27.5	2.83–2.88 ( <i>m</i> )	27.8
16	1.57–1.63 ( <i>m</i> )	41.8	1.49–1.54 ( <i>m</i> )	48.0	1.57–1.61 ( <i>m</i> )	47.9
17	4.91 ( <i>d</i> , $J=8.4$ )	100.9	4.56 ( <i>d</i> , $J=8.2$ )	100.5	4.57 ( <i>d</i> , $J=8.3$ )	100.5
18	2.16 ( <i>d</i> , $J=3.7$ )	14.0	1.38 ( <i>d</i> , $J=3.7$ )	18.4	1.33 ( <i>d</i> , $J=3.7$ )	18.4
19	3.22–3.29 ( <i>m</i> )	55.3	2.46–1.51 ( <i>m</i> )	57.5	2.59–2.63 ( <i>m</i> )	57.3
20	1.74–1.79 ( <i>m</i> )	39.0	1.60–1.63 ( <i>m</i> )	41.2	1.81–1.87 ( <i>m</i> )	38.1
21	5.38 ( <i>d</i> , $J=1.9$ )	93.8	3.54–3.60 ( <i>m</i> ), 3.48–3.51 ( <i>m</i> )	63.5	4.10–4.15 ( <i>m</i> ), 4.18–4.23 ( <i>m</i> )	66.0
17-AcO					2.08 ( <i>s</i> )	173.0, 21.0
10-MeO	3.82 ( <i>s</i> )	56.1	3.78 ( <i>s</i> )	56.3	3.82 ( <i>s</i> )	56.4
17-MeO	3.49 ( <i>s</i> )	55.4				

Fig. 2. Key  $^1\text{H}$ , $^1\text{H}$ -COSY (—) and HMBC (H $\rightarrow$ C) correlations of vinmajorine **C** (**1**)Fig. 3. Key ROESY (H $\leftrightarrow$ H) correlations of vinmajorine **C** (**1**)

H–C(21) determined by the  $^1\text{H}$ , $^1\text{H}$ -COSY correlations (Fig. 2), in combination with a series of HMBCs (Fig. 2) of H<sub>b</sub>–C(6) ( $\delta(\text{H})$  2.58, *d*,  $J=15.4$ ) with C(7) and C(8), of H–C(3) ( $\delta(\text{H})$  4.10, *d*,  $J=10.4$ ) with C(2) and C(7), and of H–C(21) ( $\delta(\text{H})$  5.38, *d*,  $J=1.9$ ) with C(17) ( $\delta(\text{C})$  100.9), further confirmed the above deduction. However, the only difference between the two compounds was that compound **1** possessed one more MeO group. The two MeO groups were located at C(10) ( $\delta(\text{C})$  55.2) and C(17) ( $\delta(\text{C})$  100.9), respectively, according to the HMBCs from one MeO group at  $\delta(\text{H})$  3.82 to C(10), and from the other MeO group at  $\delta(\text{H})$  3.49 to C(17).

The relative configuration of **1** was assigned on the basis of ROESY experiment. The NOE correlations (Fig. 3) of H–C(19) and H–C(20) with H–C(3), and of Me(18) with H–C(5) indicated that H–C(3), H–C(5), H–C(19) and H–C(20) were  $\alpha$ -oriented. The NOE cross-peaks of H–C(15) with H–C(17), of H<sub>a</sub>–C(14) with H–C(16), and of H–C(21) with Me(18) indicated the ( $R^*$ ) configuration of C(15), C(16), C(17), and C(21). Therefore, compound **1** was unambiguously established as ( $5\alpha,15\beta,16R,17\alpha,19\beta,20\alpha,21\beta$ )-10,17-dimethoxy-21-methyl-18-oxa-5,16-cyclohimban-19-ol, and named as vinmajorine **C**.

Compound **2**, obtained as white amorphous powder, possessed the molecular formula of  $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_3$  based on the HR-EI-MS ( $m/z$  340.1784 ( $M^+$ )), suggesting that **2** was an isomer of alstoyunine **A** [33]. Careful comparison of the 1D- and 2D-NMR data with those of alstoyunine **A** led to the deduction that they were similar, except for the replacement of the former MeO group by a OH group in compound **2**. The HMBC between the MeO group at  $\delta(\text{H})$  3.78 (*s*) and C(10) ( $\delta(\text{C})$  154.9 (*s*)) suggested that the MeO group should be located at C(10) rather than at C(21) as in alstoyunine **A**, which was further supported by the presence of an *ABX* coupling system at  $\delta(\text{H})$  6.89 (*d*,  $J=2.2$ , H–C(9)), 6.70 (*dd*,  $J=8.8, 2.2$ , H–C(11)), and 7.16 (*d*,  $J=8.8$ , H–C(12)) in the indole ring. Moreover, the HMBC cross-peaks from H–C(15) ( $\delta(\text{H})$  2.05–2.09 (*m*)), H–C(19) ( $\delta(\text{H})$  2.46–1.51 (*m*), and H–C(20) ( $\delta(\text{H})$  1.60–1.63 (*m*)) to the  $\text{CH}_2$  group at  $\delta(\text{C})$  63.5 assigned this  $\text{CH}_2$  group as C(21), which further confirmed the unsubstituted nature of this position.

The ROESY spectrum showed correlations of H–C(3)/H–C(19), H–C(3)/H–C(20), H–C(18)/H–C(5), H–C(15)/H–C(17) and H<sub>a</sub>–C(14)/H–C(16), which indicated that the relative configurations at C(3), C(5), C(15), C(16), C(17),

Table 2. Cytotoxic Activities of Compounds 1–3

Compound	$IC_{50}$ [ $\mu\text{M}$ ]				
	HL-60	SMMC-7721	A-549	MCF-7	SW-480
<b>1</b>	> 40	> 40	19.86	> 40	> 40
<b>2</b>	> 40	> 40	> 40	> 40	> 40
<b>3</b>	> 40	> 40	34.89	> 40	> 40
DDP <sup>a)</sup>	1.05	7.33	8.29	15.92	14.43

<sup>a)</sup> DDP = *cis*-Dichlorodiamineplatinum(II); used as positive control.

C(19), and C(20) in **2** were the same as those in alstoyunine A. Accordingly, the structure of **2** was unequivocally elucidated as (5 $\alpha$ ,15 $\beta$ ,16R,17 $\alpha$ ,20 $\alpha$ ,21 $\beta$ )-10-methoxy-21-methyl-18-oxa-5,16-cycloyohimban-17-ol, and named vinmajorine D.

Compound **3** exhibited the molecular formula  $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_4$ , as determined by its HR-EI-MS at  $m/z$  382.1894 ( $M^+$ ), corresponding to eleven degrees of unsaturation. The 1D-NMR data of **3** showed similar patterns to those of **2**, the difference being an additional AcO group ( $\delta(\text{H})$  2.08 (s);  $\delta(\text{C})$  173.0 (s) and 21.0 (q)) in **3**. The connection between the AcO group and C(17) was established by the HMBs of H–C(17) ( $\delta(\text{H})$  4.57, *d*,  $J = 8.3$ ) and Me group of the AcO group ( $\delta(\text{H})$  2.08) with the C=O group at  $\delta(\text{C})$  173.0. The ROESY data analysis showed that the relative configuration of **3** was the same as in compound **2**. Thus, the structure of compound **3** was thereby identified to be (5 $\alpha$ ,15 $\beta$ ,16R,17 $\alpha$ ,20 $\alpha$ ,21 $\beta$ )-10-methoxy-21-methyl-18-oxa-5,16-cycloyohimban-17-yl acetate, named as vinmajorine E.

Compounds **1–3** were evaluated for their cytotoxicities against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480) using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) method. The results (Table 2) revealed that compounds **1** and **3** exhibited moderate cytotoxic activities against A-549 cell with  $IC_{50}$  values of 19.86 and 34.89  $\mu\text{M}$ , respectively.

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### Experimental Part

**General.** TLC: silica gel plates  $GF_{254}$  (Qingdao Haiyang Chemical Co., Ltd., Qingdao, P. R. China). Fractions were monitored by TLC using various solvent systems, and spots were visualized by spraying improved Dragendorff's reagent to the  $\text{SiO}_2$  plates or by heating  $\text{SiO}_2$  plates sprayed with 10%  $\text{H}_2\text{SO}_4$  in EtOH. Column chromatography (CC): MCI gel (CHP 20P, 75–150  $\mu\text{m}$ ; Mitsubishi Chemical Corporation, Tokyo, Japan), Polyamide (PA, 80–100 mesh; Sinopharm Chemical Reagent Co., Ltd., Shanghai, P. R. China) and silica gel ( $\text{SiO}_2$ , 100–200 or 200–300 mesh; Qingdao Haiyang Chemical Co., Ltd., Qingdao, P. R. China). Optical rotations: Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). UV Spectra: Shimadzu UV-2401A spectrophotometer (Shimadzu, Tokyo, Japan);  $\lambda_{\text{max}}$  (log  $\epsilon$ ) in nm. IR Spectra: Tenor 27 spectrophotometer (Bruker Optics, Ettlingen,

Germany) using KBr pellets;  $\tilde{\nu}$  in  $\text{cm}^{-1}$ . 1D- and 2D-NMR spectra: Bruker AM-400, DRX-500, or AVANCE III-600 spectrometers (Bruker, Karlsruhe, Germany);  $\delta$  in ppm rel. to  $\text{Me}_4\text{Si}$  as internal standard,  $J$  in Hz. ESI-MS: Agilent 6530Q of spectrometer (Agilent, Palo Alto, CA, USA); in  $m/z$ . HR-EI-MS: Waters Auto Premier P776 spectrometer (Waters, Milford, MA, USA); in  $m/z$ .

**Plant Material.** The whole plants of *V. major* were collected in Kunming Botanical Garden, Yunnan Province, P. R. China, in September 2012, and identified by Prof. Xiao Cheng, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (120925) 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 whole plants (20 kg) of *V. major* were crushed and extracted with 90% EtOH/ $\text{H}_2\text{O}$  ( $3 \times 40\text{L}$ , 3 d, each) at r.t. to yield an extract. After removal of EtOH under reduced pressure, the residue was dissolved in 1% aq. HCl and partitioned with AcOEt for three times. The acidic soln. was subsequently basified with  $\text{NH}_3 \cdot \text{H}_2\text{O}$  to pH 9–10, and partitioned with AcOEt for three times to afford a total alkaloidal extract (100 g), which was adsorbed on polyamide and subjected to medium pressure liquid chromatography (MPLC) over MCI, eluting with MeOH/ $\text{H}_2\text{O}$  gradient system (0, 20, 30, 40, 50, 60, 70, 80, 90, and 100%, 4 l for each gradient) to give five fractions, *Frs. I–V*.

*Fr. II* (6.5 g) was further purified by  $\text{SiO}_2$  CC ( $\text{CHCl}_3/\text{MeOH}$  19:1–10:1), which gave compounds **3** (5 mg), **15** (19 mg), and **18** (8 mg), and a mixture (200 mg). The mixture was further separated by HPLC eluting with 28% MeOH/ $\text{H}_2\text{O}$  to provide compound **16** (70 mg,  $t_R = 18.5$  min). *Fr. III* (34.6 g) was subjected to  $\text{SiO}_2$  CC (petroleum ether/acetone, from 9:1 to 4:1) to give six subfractions, *Frs. III-1–III-6*. *Fr. III-4* (5.8 g) was submitted to  $\text{SiO}_2$  CC ( $\text{CHCl}_3/\text{MeOH}$ , 50:1–10:1) to yield compounds **1** (3 mg), **2** (23 mg), **6** (15 mg), **8** (10 mg), **13** (25 mg), **19** (21 mg), and **20** (4 mg). *Fr. IV* (31.6 g) was chromatographed over  $\text{SiO}_2$  (PE/acetone 8:1–1:1) to afford five subfractions, *Frs. IV-1–IV-5*. *Fr. IV-4* (4.5 g) was purified by  $\text{SiO}_2$  CC ( $\text{CHCl}_3/\text{MeOH}$  80:1–10:1) to obtain compounds **12** (8 mg), **17** (13 mg), and **21** (8 mg). *Fr. V* (16.4 g) was successively subjected to RP-18 (30% MeOH/ $\text{H}_2\text{O}$ ) and  $\text{SiO}_2$  CC (PE/acetone 15:1–1:1) to yield compounds **4** (2.1 g), **5** (22 mg), **7** (32 mg), **9** (2.8 g), **10** (46 mg), **11** (20 mg), and **14** (30 mg).

**Vinmajorine C** (= (5 $\alpha$ ,15 $\beta$ ,16R,17 $\alpha$ ,19 $\beta$ ,20 $\alpha$ ,21 $\beta$ )-10,17-Dimethoxy-21-methyl-18-oxa-5,16-cycloyohimban-19-ol; **1**). Colorless oil.  $[\alpha]_D^{20} = -11.23$  ( $c = 0.20$ , MeOH). UV (MeOH): 278 (3.87), 225 (4.40). IR (KBr): 3418, 2925, 1629, 1455, 1217, 1146, 1032.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ ): see Table 1. HR-EI-MS: 370.1899 ( $M^+$ ,  $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_4^+$ ; calc. 370.1893).

**Vinmajorine D** (= (5 $\alpha$ ,15 $\beta$ ,16R,17 $\alpha$ ,20 $\alpha$ ,21 $\beta$ )-10-Methoxy-21-methyl-18-oxa-5,16-cycloyohimban-17-ol; **2**). White, amorphous powder.  $[\alpha]_D^{20} = +64.28$  ( $c = 0.21$ , MeOH). UV (MeOH): 279 (3.84), 225 (4.33). IR (KBr): 3418, 2925, 1712, 1630, 1454, 1216, 1149, 1029.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ ): see Table 1. HR-EI-MS: 340.1784 ( $M^+$ ,  $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_3^+$ ; calc. 340.1787).

**Vinmajorine E** (= (5 $\alpha$ ,15 $\beta$ ,16R,17 $\alpha$ ,20 $\alpha$ ,21 $\beta$ )-10-Methoxy-21-methyl-18-oxa-5,16-cycloyohimban-17-yl Acetate; **3**). Colorless oil.  $[\alpha]_D^{20} =$

+33.06 ( $c=0.21$ , MeOH). UV (MeOH): 278 (3.70), 225 (4.19). IR (KBr): 3425, 2925, 1712, 1629, 1384, 1236, 1151.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ ): see Table 1. HR-EI-MS: 382.1894 ( $M^+$ ,  $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_4^+$ ; calc. 382.1893).

**Cytotoxicity Assays.** Five human tumor cell lines, MCF-7 (breast cancer), SMMC-7721 (hepatocellular carcinoma), HL-60 (human myeloid leukemia), SW480 (colon cancer), and A-549 (lung cancer) cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5%  $\text{CO}_2$  at 37°. The cytotoxicity assay was performed according to the MTT method in 96-well microplates [34]. Briefly, 100  $\mu\text{l}$  adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of  $1 \times 10^5$  cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40  $\mu\text{M}$  in triplicates for 48 h, with cisplatin (Sigma, USA) as positive control. After compound treatment, cell viability was detected and the cell growth curve was graphed. The  $\text{IC}_{50}$  values were calculated by the Reed and Muench method [35].

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