FULL PAPER

Two New Nimbolinin-Type Limonoids from the Fruits of *Melia toosendan*

by **Qiong Zhang***[a]), **Yong-Gang Zhang**[a]), **Qing-Shan Li**[a]), and **Zhi-Da Min**[b])

[a]) Department of Pharmaceutical Science, Shanxi Medical University, Taiyuan 030001, P. R. China (phone: +86-351-4690071; fax: +86-351-4690322; e-mail: zhangqiong_83@hotmail.com)

[b]) Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, P. R. China

Two new nimbolinin-type limonoids, 12-ethoxynimbolinins E and F (**1** and **2**, resp.), together with seven known analogues, 1α-benzoyloxy-3α-acetoxyl-7α-hydroxy-12β-ethoxynimbolinin (**3**), nimbolinin B (**4**), meliatoosenin L (**5**), 14,15-deoxy-11-oxohavanensin 3,12-diacetate (**6**), 12α-hydroxymeliatoosenin (**7**), toosendansin A (**8**), and toosendansin C (**9**), were isolated from the fruits of *Melia toosendan*. The structures of these compounds were elucidated by spectroscopic analysis. All the compounds were evaluated for their cytotoxicity against five tumor cell lines.

Keywords: *Melia toosendan*, Meliaceae, Limonoid, Nimbolinin, Cytotoxicity.

Introduction

Limonoids are a class of highly oxygenated tetranortriterpenoids, and occur mainly in the Meliaceae and Rutaceae families and less frequently in the Cneoraceae and Simaroubaceae in the plant kingdom. Until now, about 1300 limonoids with more than 35 carbon frameworks had been isolated [1][2]. The plant family Meliaceae is rich in structurally diverse limonoids with a variety of potentially useful biological properties, including insect antifeedant, antimicrobial, antiprotozoal, anti-inflammatory, and anticancer activities [3]. *Melia toosendan* Sieb. et Zucc. is mainly distributed in the southwest region of China [4]. The fruits of *M. toosendan* have long been known as 'Chuan-Lian-Zi' or 'Jin-Ling-Zi' in Chinese, and used as antihelmintic and analgetic reagents [4]. In recent years, the chemical constituents of the fruits of *M. toosendan* have been studied extensively. Different types of limonoids including apoeuphors, meliacins, trichilinins, nimbolinins, C(19)/C(29)-bridged acetals, ring *C*-seco limonoids, and spiro limonoids have been isolated [5][6]. As part of our ongoing search for bioactive limonoids from the fruits of *M. toosendan*, two new nimbolinin-type limonoids and seven known analogues (*Fig. 1*) were isolated from the fruits of *M. toosendan*. All the compounds were evaluated for their cytotoxicity against five tumor

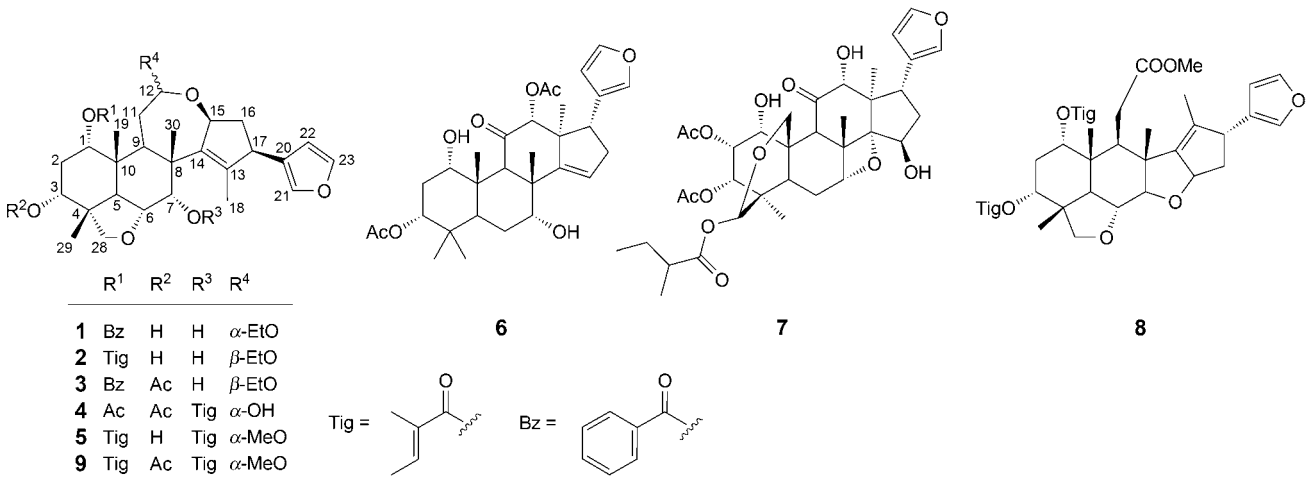

Fig. 1. Structures of compounds **1** – **9**.

 DOI: 10.1002/hlca.201500516

cell lines. Herein, we report the details of the isolation, structural elucidation, and the biological evaluation of these compounds.

Results and Discussion

Compound **1** was obtained as amorphous powder. The molecular formula was determined as $C_{35}H_{44}O_8$ by HR-ESI-MS (*m*/*z* 615.3032 [*M*+Na]$^+$; calc. 615.3036). The IR absorption bands at 1716 and 3446 cm^{-1} suggested the presence of C=O and OH groups. The ^{1}H- and ^{13}C-NMR signals (*Table 1*) of **1** were assigned by different 2D-NMR experiments. The combined analysis of its ^{1}H-, ^{13}C- and 2D-NMR spectra revealed the presence of a benzoyl group (δ(H) 8.08 (*d*, *J* = 7.2, 2 H), 7.43 (*t*, 2 H), 7.54 (*t*, 1 H); δ(C) 164.5, 130.8, 129.5, 128.4, 132.8). The ^{1}H,^{1}H-COSY spectrum indicated the presence of an EtO group (δ(H) 0.43 (*t*, *J* = 7.1, 3 H), 3.02 – 3.08 (*m*, 1 H), 2.61 – 2.65 (*m*, 1 H); δ(C) 14.3, 61.9). The HMBC (*Fig. 2*) between H–C(2′) (δ(H) 0.43 (*t*, *J* = 7.1, 3 H) and C(1′) (δ(C) 61.9) further confirmed the existence of the EtO group. A furan ring (δ(H) 6.36 (*s*), 7.23 (*s*), 7.26 (*s*); δ(C) 128.7, 138.8, 110.3, 142.6) was also apparent from the NMR spectra. Further comparison of the chemical shifts with those limonoids isolated from *M. toosendan* indicated that **1** is a nimbolinin-type limonoid [3][5][6]. The chemical shift of H–C(1), H–C(3), H–C(7), and H–C(12) were assigned as 5.09, 3.82, 4.43, and 4.67, respectively, based on the HSQC and HMBC spectra. The position of various functional groups was established through HMBC experiments. The HMBCs of H–C(1) (δ(H) 5.09 (*s*)) with C(1″) (δ(C) 164.5) and H–C(12) (δ(H) 4.67 (br. *s*)) with C (1′) (δ(C) 61.9) suggested that the benzoyl group was situated at C(1) and the EtO group was located at C(12). Accordingly, the two OH groups were placed at C(3) and C(7), respectively. The relative configuration of **1** was confirmed by the observed correlations in the NOESY spectrum (*Fig. 2*). The NOE correlations of Me(29)/H–C (3), Me(29)/H–C(6), and Me(29)/Me(19) suggested the β-orientation of H–C(3) and thus the β-orientations of Me (29), H–C(6), and Me(19). The NOE correlation of Me (19)/H–C(1) revealed that the H–C(1) was in the β-configuration. In turn, H_b–C(11) (δ(H) 1.52 – 1.58 (*m*), β-orientation) had a NOE correlation with H–C(12), which indicated that the H–C(12) was in the β-configuration. The NOE correlations between H–C(7)/H–C(6) and H–C (7)/Me(30) suggested that the H–C(7) was in the β-configuration, while the NOE correlations of H–C(15)/H–C (16α) and H–C(17)/H–C(16α) implicated an α-configuration for H–C(17). Thus, the structure of compound **1** was characterized as 1α-benzoyloxy-3α,7α-dihydroxy-12α-ethoxynimbolinin, named 12-ethoxynimbolinin E.

Compound **2** was obtained as amorphous powder. The molecular formula was determined as $C_{33}H_{46}O_8$ by HR-ESI-MS (*m*/*z* 593.3190 [*M*+Na]$^+$; calc. 593.3192). The IR spectrum showed the presence of OH (3435 cm^{-1}) and C=O (1701 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table 1*) indicated the presence of the following fragments: a tigloyl group (δ(H) 6.97 (*dd*, *J* = 1.2, 7.0, 1 H), 1.85 (*d*, *J* = 7.0, 3 H), 1.89 (*s*, 3 H); δ(C) 165.4, 138.7, 128.4, 15.0, 12.4) and an EtO group (δ(H) 1.15 (*t*, *J* = 7.1, 3 H), 3.36 – 3.42 (*m*, 1 H), 3.70 – 3.76 (*m*, 1 H); δ(C) 15.2, 63.9). Comparison of its NMR data with compound **1** indicated that they possessed a similar skeleton and the main difference was the obvious downfield shift of C(12) (from δ(C) 96.0 in **1** to 103.7 in **2**). The tigloyl group was

Table 1. ^{1}H-(400 MHz) and ^{13}C-NMR (100 MHz) data for compounds **1** – **2** in $CDCl_3$ (δ in ppm, *J* in Hz)

Position	1		2	
	δ(H)	δ(C)	δ(H)	δ(C)
1	5.09 (br. *s*)	73.1	5.07 (br. *s*)	73.0
2a	2.31 – 2.35 (*m*)	30.4	2.28 – 2.32 (*m*)	30.7
2b	2.13 – 2.17 (*m*)		1.98 – 2.02 (*m*)	
3	3.80 – 3.84 (*m*)	71.0	3.82 – 3.84 (*m*)	70.8
4		44.3		44.2
5	2.95 (*d*, *J* = 12.4)	37.3	2.71 (*d*, *J* = 12.4)	37.4
6	4.09 (*dd*, *J* = 12.4, 2.6)	73.8	4.01 (*dd*, *J* = 12.4, 2.6)	73.7
7	4.43 (*d*, *J* = 2.6)	73.4	4.37 (*d*, *J* = 2.6)	73.2
8		46.2		46.3
9	3.21 (*d*, *J* = 10.0)	34.9	2.53 (*d*, *J* = 9.6)	37.2
10		41.2		41.7
11a	1.76 – 1.84 (*m*)	32.2	1.64 – 1.70 (*m*)	33.2
11b	1.52 – 1.58 (*m*)		1.53 – 1.57 (*m*)	
12	4.67 (br. *s*)	96.0	4.04 (br. *s*)	103.7
13		138.4		142.7
14		144.4		143.4
15	4.93 (*d*, *J* = 7.6)	77.2	4.39 (*d*, *J* = 7.9)	81.8
16a	2.46 – 2.53 (*m*)	37.9	2.42 – 2.48 (*m*)	38.0
16b	1.45 – 1.49 (*m*)		1.61 – 1.67 (*m*)	
17	3.38 – 3.42 (*m*)	46.7	3.36 – 3.42 (*m*)	46.2
18	1.73 (*s*)	16.2	1.69 (*s*)	16.1
19	0.99 (*s*)	16.0	0.95 (*s*)	16.2
20		128.7		128.3
21	7.26 (br. *s*)	138.8	7.26 (br. *s*)	138.8
22	6.36 (br. *s*)	110.3	6.44 (br. *s*)	110.5
23	7.23 (br. *s*)	142.6	7.27 (br. *s*)	142.6
28a	4.12 (*d*, *J* = 7.4)	78.1	4.07 (*d*, *J* = 8.0)	78.0
28b	3.62 (*d*, *J* = 7.4)		3.60 (*d*, *J* = 8.0)	
29	1.14 (*s*)	20.2	1.11 (*s*)	19.9
30	1.34 (*s*)	20.8	1.32 (*s*)	20.9
EtO–C(12)				
1′	3.02 – 3.08 (*m*)	61.9	3.70 – 3.76 (*m*)	63.9
	2.61 – 2.65 (*m*)		3.36 – 3.42 (*m*)	
2′	0.43 (*t*, *J* = 7.1)	14.3	1.15 (*t*, *J* = 7.0)	15.2
BzO				
1″		164.5		
2″		130.8		
3″,7″	8.08 (*d*, *J* = 7.2)	129.5		
4″,6″	7.43 (*t*)	128.4		
5″	7.54 (*t*)	132.8		
TigO				
1″				165.4
2″				128.4
3″			6.97 (*dd*, *J* = 1.2, 7.0)	138.7
2″-Me			1.89 (*s*)	12.4
3″-Me			1.85 (*d*, *J* = 7.0)	15.0

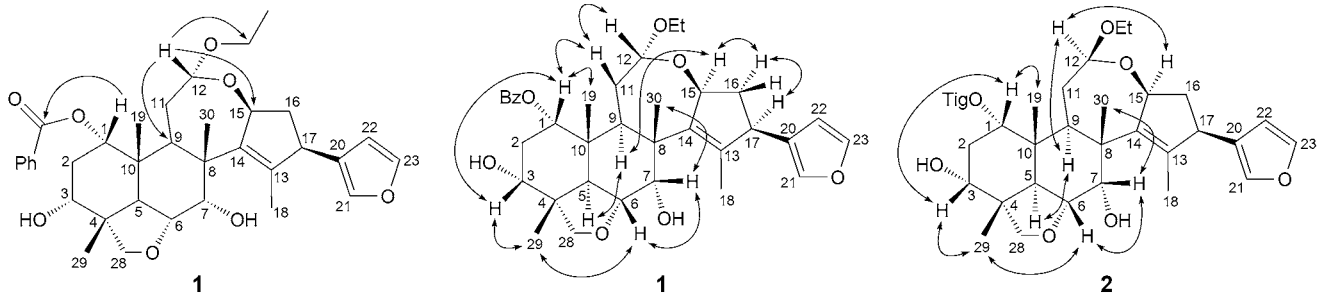

Fig. 2. Key HMBC (H→C) and NOE (H↔H) interactions of **1** and **2**.

linked to C(1) according to the HMBC spectrum. The stereochemistry of **2** was established by a NOESY experiment (*Fig. 2*). The observation of NOE effects between H–C(9)/H–C(5), H–C(9)/H–C(12), and H–C(9)/H–C(15) indicated the α-configuration of H–C(12), which deduced a significant downfield shift for C(12) (δ(C) 103.7). Therefore, the structure of compound **2** was elucidated as 1α-tigloyloxy-3α,7α-dihydroxy-12β-ethoxynimbolinin, named 12-ethoxynimbolinin F.

The known compounds were identified as 1α-benzoyloxy-3α-acetoxyl-7α-hydroxy-12β-ethoxynimbolinin (**3**) [6], nimbolinin B (**4**) [7], meliatoosenin L (**5**) [8], 14,15-deoxy-11-oxohavanensin 3,12-diacetate (**6**) [9], 12α-hydroxymeliatoosenin (**7**) [9], toosendansin A (**8**) [10], and toosendansin C (**9**) [10] by comparing their spectroscopic data with those reported. All these known compounds have been previously reported from *M. toosendan*.

Limonoids **1** – **9** were all evaluated for their cytotoxic activities against five human tumor cell lines: myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW480). However, only compounds **1**, **3**, and **7** exhibited cytotoxicity against certain tumor cell lines with the IC_{50} values in the range of 21.2 – 39.5 μM (*Table 2*).

This research was financially supported by the *National Natural Science Foundation of China* (No. 31440027), the *Scientific and Technological Innovation Programs of Higher Education Institutions in Shanxi* (No. 2014133), the *Natural Science Foundation for Young Scientists of Shanxi Province, China* (No. 2011021007-3), the *Science and Technology Innovation Found of Shanxi Medical University* (No. C01201008), and the *Program for the Top Science and Technology Innovation Teams of Higher Learning Institutions of Shanxi Province.*

Table 2. The cytotoxicity (IC_{50} [μM]) of isolated compounds **1** – **9**

Compound	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	21.5	> 40	26.4	25.2	31.8
2	> 40	> 40	> 40	> 40	> 40
3	> 40	21.2	39.5	26.1	> 40
4	> 40	> 40	> 40	> 40	> 40
5	> 40	> 40	> 40	> 40	> 40
6	> 40	> 40	> 40	> 40	> 40
7	> 40	30.7	23.0	> 40	29.8
8	> 40	> 40	> 40	> 40	> 40
9	> 40	> 40	> 40	> 40	> 40
Cisplatin[a])	1.1	4.5	6.6	13.1	11.1

[a]) Cisplatin was used as positive control.

Experimental Part

General

Column chromatography (CC): silica gel (SiO_2; *Merck*, Darmstadt, Germany) and *Sephadex LH-20* (*Pharmacia*, Uppsala, Sweden). Prep. HPLC: *Waters Prep LC 4000* system with a UV detector and an *X-bridge C-18* column (19 × 150 mm, 5 μm; *Waters*, Milford, MA, USA). Fractions were visualized by heating silica gel plates sprayed with 4-(dimethylamino)benzaldehyde. Optimal rotations: *PerkinElmer PE 241* polarimeter (*PerkinElmer*, Fremont, CA, USA). IR Spectra: *PerkinElmer 16 PC* FT-IR spectrophotometer (*PerkinElmer*, Fremont, CA, USA). 1D- and 2D-NMR spectra: *Bruker AV400* spectrometer (*Bruker*, Karlsruhe, Germany), with TMS as an internal standard. HR-ESI-MS: *PE Biosystems Mariner System 5140 LC/MS* spectrometer (*PerkinElmer*, Fremont, CA, USA).

Plant Material

The dried fruits of *M. toosendan* were collected from Wanxian, Sichuan Province, P. R. China, in July 2010, and identified by Prof. *M.-J. Qin* (Department of Natural Medicinal Resources, China Pharmaceutical University). A voucher specimen (No. 28-78-56-3) was deposited with the herbarium of China Pharmaceutical University.

Extraction and Isolation

The air-dried fruits of *M. toosendan* (20 kg) were crushed and extracted with AcOEt (2 h each) under reflux three times. All the extracts were combined and concentrated under vacuum to give a residue (*Fr. A*, 300 g). *Fr. A* was

subjected to SiO_2 CC (13 × 85 cm, 230 – 400 mesh), eluted with a gradient of petroleum ether (PE)/AcOEt (9:1, 8:2, 7:3, and 6:4) to afford 20 fractions (*Frs. 1 – 20*). *Fr. 9* (8 g) was further divided into five subfractions (*Frs. 9.1 – 9.5*) by SiO_2 CC (4 × 42 cm, 230 – 400 mesh), using $CHCl_3$/Me_2CO (100:1, 100:2, and 100:4) as the eluent. *Fr. 9.3* (2.7 g) was further chromatographed on SiO_2 CC (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of PE/Me_2CO (90:10, 88:12, 85:15, and 80:20) to afford seven subfractions, *Frs. 9.3.1 – 9.3.7*. *Fr. 9.3.4* (0.5 g) was chromatographed on *Sephadex LH-20* (2 × 80 cm, $CHCl_3$/MeOH 1:1) to give five subfractions (*Frs. 9.3.4.1 – 9.3.4.5)*. *Fr. 9.3.4.2* (70 mg) was purified by reversed-phase prep. HPLC using a gradient of increasing MeCN (60 – 90%) in H_2O at 18 ml/min for 20 min to give **8** (t_R = 15.7 min, 20 mg) and **9** (t_R = 12.4 min, 13 mg). *Fr. 12* (13 g) was chromatographed over SiO_2 CC (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of $CHCl_3$/Me_2CO (100:1, 100:3, and 100:5) to afford seven subfractions, *Frs. 12.1 – 12.7*. *Fr. 12.5* (62 mg) was purified by reversed-phase prep. HPLC using a gradient of increasing MeCN (55 – 65%) in H_2O at 18 ml/min for 20 min to give **5** (t_R = 11.2 min, 18 mg). *Fr. 12.7* (135 mg) was purified by reversed-phase prep. HPLC using a gradient of increasing MeCN (50 – 60%) in H_2O at 18 ml/min for 20 min to give **6** (t_R = 13.4 min, 21 mg). *Frs. 13* and *14* (11 g) were chromatographed on SiO_2 CC (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of $CHCl_3$/Me_2CO (100:2, 100:4, 100:6, and 100:8) to afford *Frs. 13.1 – 13.7*. *Fr. 13.6* (1.8 g) was chromatographed over SiO_2 CC (3 × 39 cm, 230 – 400 mesh), eluting with a gradient of PE/Me_2CO (80:20, 75:25, 70:30, and 60:40) to afford *Frs. 13.6.1 – 13.6.7*. *Fr. 13.6.3* (120 mg) was purified by reversed-phase prep. HPLC using a gradient of increasing MeCN (45 – 55%) in H_2O at 18 ml/min for 20 min to yield **3** (t_R = 9.7 min, 8 mg) and **4** (t_R = 11.5 min, 28 mg). *Fr. 13.6.6* (53 mg) was purified by reversed-phase prep. HPLC using a gradient of increasing MeCN (40 – 50%) in H_2O at 18 ml/min for 20 min to yield **1** (t_R = 10.4 min, 15 mg). *Frs. 15* and *16* (40 g) were chromatographed on SiO_2 (8 × 50 cm, 230 – 400 mesh), eluting with a gradient of $CHCl_3$/Me_2CO (100:7, 100:10, 100:15, 100:30, and 100:50) to afford *Frs. 15.1 – 15.8*. *Fr. 15.4* (10 g) was chromatographed on SiO_2 (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of PE/Me_2CO (80:20, 75:25, 70:30, and 60:40) to afford *Frs. 15.4.1 – 15.4.7*. *Fr. 15.4.4* (700 mg) was chromatographed on SiO_2 (2 × 33 cm, 230 – 400 mesh), eluting with $CHCl_3$/Me_2CO (100:8) to afford *Frs. 15.4.4.1 – 15.4.4.5*. *Frs. 15.4.4.3* and *15.4.4.4* (150 mg) were purified by reversed-phase prep. HPLC using a gradient of increasing MeCN (45 – 60%) in H_2O at 18 ml/min for 20 min to give **2** (t_R = 12.0 min, 12 mg). *Fr. 15.6* (7.3 g) was chromatographed on SiO_2 (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of PE/Me_2CO (75:25, 70:30 and 60:40) to afford *Frs. 15.6.1 – 15.6.8*. *Fr. 15.6.3* (48 mg) were purified by reversed-phase prep. HPLC using a gradient of increasing MeCN (40 – 55%) in H_2O at 18 ml/min for 20 min to give **7** (t_R = 11.2 min, 16 mg).

12-Ethoxynimbolinin E (= **1α-Benzoyloxy-3α,7α-dihydroxy-12α-ethoxynimbolinin** = **(2*R*,3a*S*,5*R*,6b*R*,7*S*,9*R*,9a*R*,11a*R*,12*S*,12a*R*)-5-Ethoxy-2-(furan-3-yl)-9,12-dihydroxy-1,6b,9a,12a-tetramethyl-3,3a,6,6a,6b,7,8,9,9a,10,11a,11b,12,12a-tetradecahydro-2*H*,5*H*-cyclopenta[*b*]furo[2′,3′,4′:4,5]naphtho[2,1-*d*]oxepin-7-yl benzoate**; **1**). Amorphous powder. $[\alpha]_D^{25}$ = 28.3 (*c* = 0.09, $CHCl_3$). IR (KBr): 3446, 2924, 1716, 1459, 1220, 772. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 615.3032 ($[M+Na]^+$, $C_{35}H_{44}NaO_8^+$; calc. 615.3036).

12-Ethoxynimbolinin F (= **1α-Tigloyloxy-3α,7α-dihydroxy-12β-ethoxynimbolinin**= **(2*R*,3a*S*,5*S*,6b*R*,7*S*,9*R*,9a*R*,11a*R*,12*S*,12a*R*)-5-Ethoxy-2-(furan-3-yl)-9,12-dihydroxy-1,6b,9a,12a-tetramethyl-3,3a,6,6a,6b,7,8,9,9a,10,11a,11b,12,12a-tetradecahydro-2*H*,5*H*-cyclopenta[*b*]furo[2′,3′,4′:4,5]naphtho[2,1-*d*]oxepin-7-yl (2*E*)-2-Methylbut-2-enoate**; **2**). Amorphous powder. $[\alpha]_D^{25}$ = +9.3 (*c* = 0.11, $CHCl_3$). IR (KBr): 3435, 2928, 1701, 1262, 1073, 601. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 593.3190 ($[M+Na]^+$, $C_{33}H_{46}NaO_8^+$; calc. 593.3192).

Cytotoxicity Assay

The MTT method [11] was used for assessing the cytotoxicity of all isolated compounds against the five tumor cell lines (HL-60 human myeloid leukemia, SMMC-7721 hepatocellular carcinoma, A-549 lung cancer, MCF-7 breast cancer, and SW480 colon cancer) with cisplatin as the positive control.

REFERENCES

[1] X. Fang, Y. T. Di, X. J. Hao, *Curr. Org. Chem.* **2011**, *15*, 1363.
[2] H. Zhang, J. Tan, D. VanDerveer, X. Wang, M. J. Wargovich, F. Chen, *Phytochemistry* **2009**, *70*, 294.
[3] Q.-G. Tan, X.-D. Luo, *Chem. Rev.* **2011**, *111*, 7437.
[4] 'Flora Republicae Popularis Sinicae', Science Press, Beijing, 1997, Vol. 43, p. 102.
[5] Y. Zhang, C.-P. Tang, C.-Q. Ke, S. Yao, Y. Ye, *J. Nat. Prod.* **2010**, *73*, 664.
[6] Q. Zhang, J.-K. Li, R. Ge, J.-Y. Liang, Q.-S. Li, Z.-D. Min, *Fitoterapia* **2013**, *90*, 192.
[7] W. Kraus, M. Bokel, *Chem. Ber.* **1981**, *114*, 267.
[8] Y. Zhang, C.-P. Tang, C.-Q. Ke, X.-Q. Li, H. Xie, Y. Ye, *Phytochemistry* **2012**, *73*, 106.
[9] G.-Y. Zhu, L.-P. Bai, L. Liu, Z.-H. Jiang, *Phytochemistry* **2014**, *107*, 175.
[10] L. Chen, J.-X. Zhang, B. Wang, S.-Z. Mu, X.-J. Hao, *Fitoterapia* **2014**, *97*, 204.
[11] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.

Received December 8, 2015
Accepted February 24, 2016

