

## Cytotoxic Macroyclic Diterpenoids from *Euphorbia helioscopia*

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A new cytotoxic macrocyclic diterpenoid, euphornin L (**1**), together with seven known analogues were isolated from the plant *Euphorbia helioscopia* L. The structure of **1** was elucidated by spectral data and X-ray crystallographic analysis. Euphornin L (**1**) and euphoscin F (**2**) exhibited significant cytotoxicity against HL-60 cell lines with IC<sub>50</sub> values of 2.7 and 9.0 μM, respectively. The <sup>13</sup>C-NMR data of euphoscin F (**2**), epieuphoscin B (**3**), euphoscin B (**5**), and euphoscin C (**6**) were also reported for the first time.

**Key words:** Diterpenoid, Euphornin L, X-ray, *Euphorbia helioscopia* L, Cytotoxicity

## INTRODUCTION

The genus *Euphorbia* (Euphorbiaceae) mainly contains diterpenoid compounds that are responsible for the skin-irritating, tumor-promoting, and cytotoxic activities (Singla and Kamalal, 1990; Amir, 2006). *Euphorbia helioscopia* L. has been used to treat malaria, bacillary dysentery, osteomyelitis, and tumor in Chinese folk medicine (Hua et al., 1999; Gao, 1997). Up to now, only one obvious cytotoxic macrocyclic diterpenoid ester has been reported from this plant during the past three decades (Lu et al., 2008). Bioassay-guided phytochemical study on *E. helioscopia* revealed a new cytotoxic macrocyclic diterpenoid ester (**1**), together with seven known analogues (**2-8**). Compounds **1** and **2** exhibited significant cytotoxicity against HL-60 cell lines with IC<sub>50</sub> values of 2.7 and 9.0 μM, respectively. Since there were no reports in the literature, <sup>13</sup>C-NMR data of compounds **2**, **3**, **5**, and **6** were also reported in this paper.

## MATERIALS AND METHODS

### General experimental procedures

Melting points were determined on a Fisher-Johns apparatus and uncorrected. Optical rotations were obtained

on a JASCO P-1020 digital polarimeter. UV spectra were recorded on Beckmen DU® 640 spectrophotometer. IR spectra were recorded using a Bruker model. 1D-NMR and 2D-NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard and chemical shifts were recorded as δ values. ESI-MS was measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. Semipreparative HPLC was performed using ODS columns [YMC-pack ODS-A, 10 × 250 mm, 5 μm, 4.0 mL/min] in Waters 600 multisolvent delivery system equipped with a photodiode array detector (Waters, 996).

### Plant material

The herbal drug “maoyancao” (*E. helioscopia*) was purchased as dried whole plants from a drug store in Henan Province of China. Botanical identification was carried out by Dr Yan Luo and the voucher specimen (Y.LUO613) was deposited in the College of Marine Life, Ocean University of China.

### Extraction and isolation

The dried whole plant (4.5 kg) of *E. helioscopia* was powdered and extracted with 95% EtOH (3 × 10 L) to give a dark green residue (140.6 g) after removal of the solvent. The residue was distributed between light petroleum ether (3 L) and water (3 L) for 3 times, and the light petroleum ether layers afforded green oil (71.0 g) after removing the solvent. The green oil was subjected to column chromatography (CC, 10 × 50 cm) over macro-

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porous resin (AB-8) and eluted with 50% EtOH (20 L), 80% EtOH (15 L) and 95% EtOH (15 L) successively. The 80% EtOH elutions (15.0 g) were chromatographed on silica gel (300–400 mesh, 250 g) and gradiently eluted with petroleum ether-CHCl<sub>3</sub> and CHCl<sub>3</sub>-MeOH to divide into 11 fractions (Fraction 1–11). Fraction 8 (2.8 g) was separated into 12 sub-fractions by CC on ODS (30 g) eluting with MeOH-H<sub>2</sub>O solutions (30–90%). Sub-fraction 5 gave compounds **3** (40.0 mg) and **5** (15.2 mg) after being subjected to CC on Sephadex LH-20 eluted with MeOH and HPLC eluted with 65% MeOH-H<sub>2</sub>O. Compounds **1** (25.0 mg) and **2** (12.5 mg) were obtained from sub-fraction 6 through CC on Sephadex LH-20, silica gel and HPLC in sequence. Fraction 9 (2.5 g) was separated into 9 sub-fractions on ODS (28 g) and eluted with MeOH-H<sub>2</sub>O solutions (40–90%). Being chromatographed over sephadex LH-20, silica gel and HPLC, sub-fraction 5 and 6 afforded compounds **4** (32.0 mg) and **8** (60.0 mg), respectively. Compound **6** (50.2 mg) was obtained from sub-fraction 8 through CC on Sephadex LH-20 and silica gel. Fraction 11 (1.5 g) was subjected to CC over ODS, sephadex LH-20, silica gel, and HPLC to give compound **7** (35.0 mg).

**Euphornin L (1):** colorless crystal (MeOH): m.p. 188–190°C;  $[\alpha]_D^{20}$  -108.2° (*c* 0.3, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 225 (3.8), 273 (2.9) nm; IR (KBr)  $\nu_{\text{max}}$  2959, 1736, 1244, 750, 705 cm<sup>-1</sup>; <sup>1</sup>H- and <sup>13</sup>C-NMR (see Table I); HRESI-MS *m/z* 649.3008 [M + Na]<sup>+</sup> (calcd. 649.2989 for C<sub>35</sub>H<sub>46</sub>O<sub>10</sub>Na).

### X-ray crystal Structure of 1

The X-ray-diffraction data of compound **1** were collected on a Siemens SMART-CCD area-detector diffractometer using the  $\varphi$ - and  $\omega$ -scan technique (scan width 1.58–25.01°;  $2\theta \leq 50^\circ$ ). A colorless prism of compound **1** (0.56 × 0.45 × 0.41 mm) obtained from MeOH was selected for

data collection, and the structure was solved by direct methods and refined by block-matrix least-squares procedure, using the SHELX-97 program system. H-positions were found from difference Fourier maps and geometric calculations. Crystal data: C<sub>35</sub>H<sub>46</sub>O<sub>10</sub>; *M*<sub>r</sub> 626.72 g mol<sup>-1</sup>; absorption coefficient 0.083 mm<sup>-1</sup>; space group *P*(2)1; triclinic, *a* = 10.9176(18), *b* = 18.069(3), *c* = 18.451(3) Å,  $\alpha$  = 90,  $\beta$  = 94.279 (2),  $\gamma$  = 90°; *V* = 3629.7(9) Å<sup>3</sup>; *Z* = 4; *F*(000) = 1344; *D*<sub>calc</sub> = 1.147 g cm<sup>-3</sup>, *T*<sub>min</sub>/*T*<sub>max</sub>: 0.9548/0.9666; data/restraints/ parameters 10586/1/812; goodness of fit on *F*<sup>2</sup> : 1.002; *R*<sub>1</sub> and *wR*<sub>2</sub> [*I*>2σ(*I*)]: 0.0560 and 0.1187, resp.; *R*<sub>1</sub> and *wR*<sub>2</sub> (all data): 0.1316 and 0.1353, resp. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 695426. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk]

### Cytotoxicity assay

The cytotoxicities of the isolates were evaluated using the MTT method (Mosmann, 1983) with HL-60 cell lines, and SRB method (Skehan et al., 1990) with A-549 cell lines.

## RESULTS AND DISCUSSION

By analyzing their spectroscopic and physicochemical properties, compounds **2–8** were identified as euphoscin F, epieuphoscin B (Yamamura et al., 1989), euphoscin A, euphoscin B (Yamamura et al., 1981), euphoscin C (Yamamura et al., 1989), euphoheliosnioid A (Zhang and Guo, 2005), and euphornin (Yamamura et al., 1989) (Fig. 1).

Compound **1** was obtained as colorless prism (MeOH)

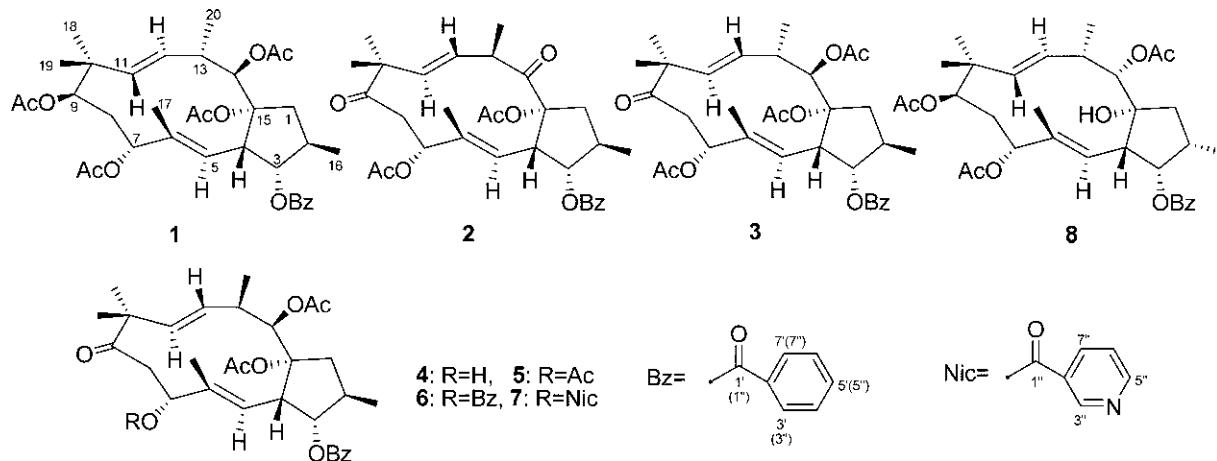


Fig. 1. The structures of compounds **1–8**

**Table I.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of **1**, **2**, **3**, **5** and **6** in  $\text{CDCl}_3$ <sup>a</sup>

Position	<b>1</b>		<b>2</b>		<b>3</b>		<b>5</b>		<b>6</b> <sup>b</sup>	
	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$								
1	2.66 (dd, 15.1, 6.6), 1.51 (dd, 15.1, 9.6)	39.7t	3.07 (m), 2.25 (m)	43.5t	2.92 (dd, 14.8, 3.2), 1.46 (dd, 14.8, 9.6)	41.1t	2.98 (dd, 15.5, 7.7), 1.43 (dd, 15.5, 9.0)	43.0t	3.01 (dd, 15.5, 8.4), 1.50 (dd, 15.5, 7.8)	43.6t
2	2.40 (m)	36.5d	2.26 (m)	39.2d	2.42 (m)	37.2d	2.45 (m)	37.7d	2.46 (m)	38.0d
3	4.78 (dd, 7.8, 7.3)	82.8d	5.19 (dd, 7.0, 2.3)	83.4d	5.00 (dd, 7.7, 5.1)	82.5d	5.19 (dd, 7.7, 3.3)	82.3d	5.13 (dd, 6.4, 2.3)	82.3d
4	3.58 (dd, 11.0, 7.8)	43.4d	3.44 (dd, 9.4, 7.0)	45.5d	3.32 (dd, 9.3, 7.7)	45.0d	3.26 (dd, 8.5, 7.7)	43.9d	3.30 (dd, 8.7, 6.4)	43.9d
5	5.73 (d, 11.5)	121.2d	5.70 (dd, 9.4, 1.4)	119.3d	5.67 (brd, 9.3)	122.4d	5.67 (brd, 8.5)	122.6d	5.87 (brd, 8.7)	122.7d
6	/	132.5s	/	139.9s	/	135.5s	/	135.8s	/	135.4s
7	4.85 <sup>c</sup>	73.0d	5.26 (dd, 11.1, 4.9)	73.2d	5.08 (dd, 9.6, 3.2)	73.6d	5.39 (dd, 11.5, 4.4)	73.4d	5.70 (dd, 11.5, 4.1)	74.1d
8	1.92 (2H, m)	32.2t	3.10 (dd, 15.1, 11.1) 2.70 (dd, 15.1, 4.9)	42.5t	2.93(brd, 14.1) 2.73 (dd, 14.1, 3.2)	39.8t	3.14 (dd, 16.0, 11.5) 2.68 (dd, 16.0, 4.4)	42.9t	3.32 (dd, 15.8, 11.5) 2.86 (dd, 15.8, 4.1)	42.8t
9	4.85 <sup>c</sup>	73.8d	/	206.6s	/	207.1s	/	207.5s	/	207.5s
10	/	39.5s	/	49.2s	/	50.3s	/	49.0s	/	49.1s
11	4.96 (d, 15.1)	135.9d	5.53 (d, 15.5)	136.3d	5.25 (d, 15.7)	132.2d	5.36 (d, 16.0)	133.7d	5.39 (d, 16.0)	133.7d
12	5.23 (dd, 15.6, 9.1)	129.2d	5.03 (dd, 15.5, 9.7)	132.3d	5.34 (dd, 15.7, 7.7)	132.1d	5.17 (dd, 16.0, 8.8)	132.8d	5.22 (dd, 16.0, 8.7)	132.4d
13	2.30 (m)	41.8d	2.66 (m)	51.4d	2.29 (m)	40.0d	2.17 (m)	37.6d	2.13 (m)	37.8d
14	6.05 (d, 10.6)	73.9d	/	211.6s	5.87(d, 9.0)	74.3d	5.93 (br.s)	75.3d	5.95 (br.s)	75.6d
15	/	92.1s	/	95.9s	/	93.0s	/	92.3s	/	92.4s
16	1.07 (3H, d, 7.0)	17.0q	1.14 (3H, d, 6.8)	18.8q	1.01 (3H, d, 7.0)	17.9q	0.92 (3H, d, 7.3)	18.9q	0.93 (3H, d, 7.3)	19.3q
17	1.67 (3H, d, 0.9)	15.7q	1.67 (3H, d, 1.3)	18.7q	1.73 (3H, d, 0.7)	17.4q	1.86 (3H, d, 0.9)	18.8q	1.95 (3H, d, 0.9)	18.8q
18	0.89 (3H, s)	22.5q	1.10 (3H, s)	25.1q	1.12 (3H, s)	24.5q	1.09 (3H, s)	25.3q	1.14 (3H, s)	25.5q
19	0.88 (3H, s)	20.1q	1.22 (3H, s)	20.1q	1.18 (3H, s)	22.6q	1.24 (3H, s)	24.9q	1.31 (3H, s)	24.9q
20	0.92 (3H, d, 6.8)	21.2q	1.22 (3H, d, 6.8)	22.0q	1.12 (3H, d, 6.4)	19.4q	1.10 (3H, d, 7.3)	22.9q	1.09 (3H, d, 7.3)	22.9q
3-OBz-1'	/	166.1s	/	165.2s	/	165.7s	/	165.4s	/	165.9s
Bz(2')	/	130.4s	/	130.5s	/	130.4s	/	130.6s	/	130.4s
Bz(3', 7')	7.96 (2H, br.d, 7.8)	129.3d	8.04 (2H, dd, 7.8, 1.2)	129.5d	7.95 (2H, br.d, 7.7)	129.4d	8.00 (2H, br.d, 7.1)	129.4d	7.86 (2H, br.d, 8.3)	129.3d
Bz(4', 6')	7.41 (2H, dd, 7.8, 7.3)	128.3d	7.46 (2H, t, 7.8)	128.4d	7.41 (2H, t, 7.7)	128.2d	7.43 (2H, t, 7.1)	128.3d	7.29 (2H, dd, 8.3, 7.3)	128.2d
Bz(5')	7.53 (t, 7.3)	132.9d	7.56 (dd, 7.8, 1.4)	132.9d	7.53 (t, 7.7)	132.8d	7.54 (t, 7.1)	133.6d	7.46 (t, 7.3)	133.6d
7-OCOCH <sub>3</sub>	1.53 (3H, s)	20.5q	1.29 (3H, s)	19.0q	1.52 (3H, s)	20.4q	1.26 (3H, s)	20.6q	/	/
7-OCOCH <sub>3</sub>	/	169.3s	/	169.3s	/	169.3s	/	169.9s	/	/
9-OCOCH <sub>3</sub>	1.98 (3H, s)	21.1q	/	/	/	/	/	/	/	/
9-OCOCH <sub>3</sub>	/	169.5s	/	/	/	/	/	/	/	/
14-OCOCH <sub>3</sub>	2.17 (3H, s)	21.3q	/	/	2.15 (3H, s)	21.1q	2.17 (3H, s)	21.0q	2.17 (3H, s)	21.1q
14-OCOCH <sub>3</sub>	/	169.9s	/	/	/	169.7s	/	170.2s	/	170.0s
15-OCOCH <sub>3</sub>	2.29 (3H, s)	22.7q	2.36 (3H, s)	21.9q	2.16 (3H, s)	21.6q	2.26 (3H, s)	22.0q	2.21 (3H, s)	22.1q
15-OCOCH <sub>3</sub>	/	170.4s	/	170.8s	/	170.0s	/	170.0s	/	170.0s

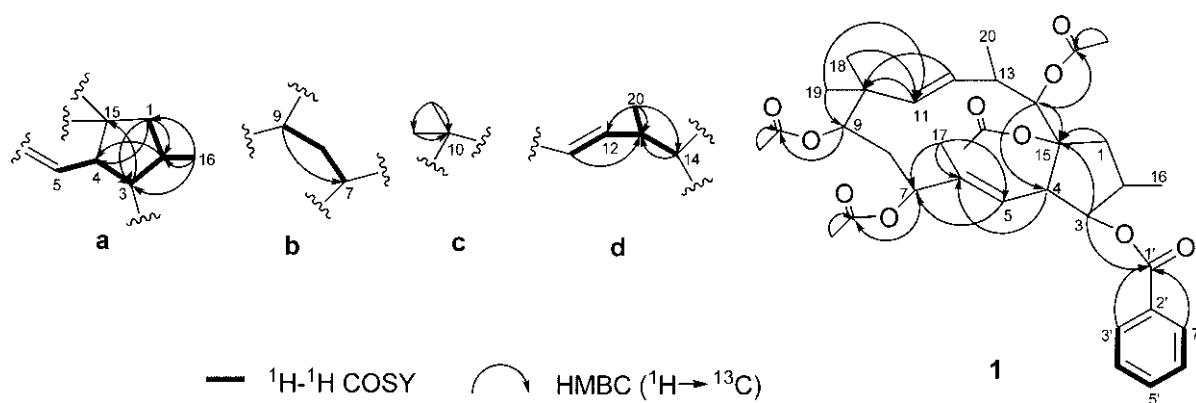
<sup>a</sup>  $^1\text{H}$ - and  $^{13}\text{C}$ - NMR spectra were obtained at 600 and 150 MHz, respectively. Unless otherwise indicated, all proton signals integrate to 1 H.

<sup>b</sup>  $^1\text{H}$ - and  $^{13}\text{C}$ - NMR data of 7-OBz were 7.57 (2H, br.d,  $J = 8.3$ , H-3", 7"), 6.97 (2H, dd,  $J = 8.3$ , 7.3, H-4", 6") and 7.31 (1H, t,  $J = 7.3$ , H-5"), and 165.4 (s, C-1"), 130.0 (s, C-2"), 129.2 (d, C-3", 7") and 127.9 (d, C-4", 6") and 132.7 (d, C-5"), respectively.

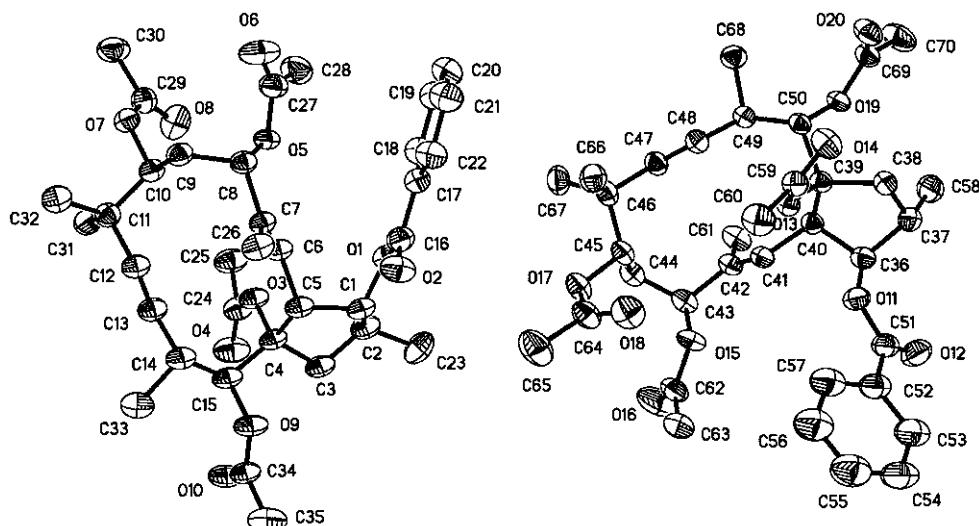
<sup>c</sup> the overlap signals.

with the specific rotation of  $[\alpha]_D^{20} -108.2^\circ$  ( $c$  0.3,  $\text{CHCl}_3$ ). Its molecular formula was established as  $\text{C}_{35}\text{H}_{46}\text{O}_{10}$  by the positive HRESI-MS quasi-molecular ion at  $m/z$  649.3008 ( $[\text{M} + \text{Na}]^+$ , calc. 649.2989). IR absorption band at 1736  $\text{cm}^{-1}$  showed the existence of ester carbonyl group. Apart from substitution of an oxygenated methylene for a carbonyl group and an additional acetoxy group, its 1D NMR data were similar to those of compound **3** (Table I), suggesting compound **1** as a benzyloxy-substituted jatrophane diterpenoid tetraacetate (Yamamura et al., 1989).  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations allowed the

construction of four structural moieties **a-d** (Fig. 2). The key HMBC correlations from H-17 to C-5, C-6, and C-7, together with the correlations between H-4 and C-6, and between H-5 and C-7 showed that **a** and **b** was connected together through the structural moiety  $=\text{C}(\text{CH}_3)-$ . The connectivity of **b** with **d** via C-10 in **c** was supported by the HMBC correlations between H-19 with C-9 and C-11, and between H-12 with C-10. And the diterpenoid skeleton was constructed completely via the  $\text{C}_{14}\text{-C}_{15}$  single bond between **a** and **d** by HMBC correlations between H-1 and H-14 with C-15, and between H-14 with C-4 (Table I and



**Fig. 2.** The key HMBC,  $^1\text{H}$ - $^1\text{H}$  COSY correlations of **1**



**Fig. 3.** X-ray crystal structure of **1**

Fig. 2). The linkage of benzyloxy and three acetoxy groups to C-3, C-7, C-9, C-14 were confirmed by corresponding HMBC correlations, respectively. The final acetoxy group was fixed to C-15 (Fig. 2). The relative configuration of compound **1** was dissolved by X-ray crystal diffraction (Fig. 3). On account of the angular proton, H-4, was biogenetically  $\beta$ -oriented, i.e. S-configuration of C-4 (Shizuri et al., 1984; Yamamura et al., 1981; Yamamura et al., 1989), the absolute configuration of compound **1** was established as 3S,4S,5E,7R,9R,11E,13S,14R,15R (Fig. 1).

The cytotoxicities of compounds **1-8** were assayed using the HL-60 cells by MTT method, and A-549 cells by SRB method. And VP-16 (etoposide) was used as the positive control with IC<sub>50</sub> values of 0.04 and 0.63  $\mu\text{M}$ , respectively. Compounds **1** and **2** exhibited cytotoxicity against HL-60 with IC<sub>50</sub> values of 2.7 and 9.0  $\mu\text{M}$ , respectively, while compounds **3-8** were inactive (IC<sub>50</sub> > 100  $\mu\text{M}$ ).

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## REFERENCES

- Amir, R. J., Chemistry and biological activity of secondary metabolites in *Euphorbia* from Iran, *Phytochemistry*, 67, 1977-1984 (2006).
- Gao, Z. L., Treatments on early-stage liver cancer mainly with large doses of *Euphorbia helioscopia*, *Jiangsu Chinese Medicine*, 11, 28 (1997).
- Hua, Y. X., Liu, S. F., and Yang, Z. Q., *Chinese Bencao*;

- Shanghai Science and Techology Press: Shanghai, 4, 782-785 (1999).
- Lu, Z-Q., Guan, S-H., Li, X-N., Chen, G-T., Zhang, J-Q., Huang, H-L., Liu, X., and Guo, D-A., Cytotoxic diterpenoids from *Euphorbia helioscopia*. *J. Nat. Prod.*, 71, 873-876 (2008).
- Mosmann, T. J., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*, 65, 55-63 (1983).
- Shizuri, Y., Kosemura, S., Ohtsuka, J., Terada, Y., Yamamura, S., Ohba, S., Ito, M., and Saito, Y., Structural and conformational studies on euphornin and related diterpenes, *Tetrahedron Lett.*, 25, 1155-1158 (1984).
- Singla, A. K. and Kamala, P., Phytoco, *Fitoterapia*, 61, 483-516 (1990).
- Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R. New colorimetric cytotoxicity assay for anticancer drug screening. *J. Nat. Cancer Inst.*, 82, 1107-1112 (1990).
- Yamamura, S., Kosemura, S., Ohba, S., Ito, M., and Saito, Y., The isolation and structures of euphoscopins A and B. *Tetrahedron Lett.*, 22, 5315-5318 (1981).
- Yamamura, S., Shizuri, Y., Kosemura, S., Ohtsuka, J., Tayama, T., Ohba, S., Ito, M., Saito, Y., and Terada, Y., Diterpenes from *Euphorbia helioscopia*. *Phytochemistry*, 28, 3421-3436 (1989).
- Zhang, W. and Guo, Y-W., Three new jatrophe-type diterpenoids from *Euphorbia helioscopia*. *Planta Med.*, 71, 283-286 (2005).