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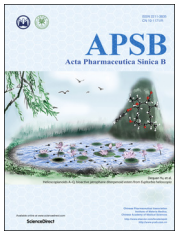

ORIGINAL ARTICLE

Helioscopianoids A–Q, bioactive jatrophane diterpenoid esters from *Euphorbia helioscopia*

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KEY WORDS

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P-glycoprotein (ABCB1) inhibitory activity;
Neuroprotective effect

Abstract The EtOH extracts of the whole plants of *Euphorbia helioscopia* afforded 17 new jatrophane diterpenoid esters, helioscopianoids A–Q (**1**–**17**), along with eight known compounds (**18**–**25**). Their structures were elucidated by extensive spectroscopic methods and $Mo_2(OAc)_4$-induced ECD analysis, and the structures of compounds **1**, **2**, and **7** were confirmed by X-ray crystallography. Compounds **1**–**17** were evaluated for inhibitory effects on P-glycoprotein (P-gp) in an adriamycin (ADM)-resistant human breast adenocarcinoma cell line (MCF-7/ADR) and neuroprotective effects against serum deprivation-induced and rotenone-induced PC12 cell damage. Compounds **8** and **16** increased the accumulation of ADM in MCF-7/ADR cells by approximately 3-fold at a concentration of 20 μmol/L. Compound **8** could attenuate rotenone-induced PC12 cell damage, and compounds **2**, **8**, and **12** showed neuroprotective activities against serum deprivation-induced PC12 cell damage.

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1. Introduction

To date, nearly 20 classes of lower diterpenoids, such as casbanes, jatrophanes, lathyranes, tiglianes, ingenanes, and daphnanes, with different core frameworks have been isolated and structurally characterized from the genus *Euphorbia*[1,2]. Among these compounds, jatrophane diterpenoids, a class of macrocyclic diterpenoids, have a unique *trans*-bicyclo[10.3.0]pentadecane framework and are generally substituted with various acyl groups (*e.g.*, benzoyl, acetyl, nicotinoyl, butanoyl and isobutyryl). Meanwhile, jatrophanes exert a wide variety of biological activities, such as the ability to modulate of P-glycoprotein (ABCB1)[3–6] as well as antiproliferative[7,8], antimalarial[9], anti-inflammatory[10], and antiviral[11] activities. From a biosynthetic perspective, jatrophanes are regarded as precursors to presegetanes, segetanes, paralianes, and pepluanes because these diterpenoids can be derived from an intramolecular cyclization of the 12-membered macrocycle of the jatrophane diterpenoids[10,12,13].

Euphorbia helioscopia L., an annual herb, belongs to the genus *Euphorbia* (Euphorbiaceae) and has been extensively used as a Chinese folk medicine for the treatments of malaria, bacillary dysentery, osteomyelitis, and tumors[14]. *E. heliosocpia* was reported to be a rich source of jatrophane diterpenoids, which are known for being highly oxygenated and containing many stereocenters[15–18]. During our efforts to systematically search for pharmacologically active diterpenoids from this poisonous traditional Chinese medicine, 17 new jatrophane diterpenoids (helioscopianoids A–Q, **1**–**17**) were characterized along with eight known analogues (**18**–**25**, Fig. 1). Herein, compounds **1**–**17** were characterized based on their physical data, especially X-ray diffraction data, and they were tested for P-gp inhibitory and neuroprotective activities.

2. Results and discussion

Compound **1** was obtained as colorless crystals and was found to have a molecular formula of $C_{29}H_{38}O_7$ based on its (+)-HR-ESI-MS ion at *m/z* 521.2528 $[M + Na]^+$ (Calcd. for $C_{29}H_{38}O_7Na$, 521.2510), corresponding to eleven indices of hydrogen deficiency. Its IR spectrum suggested the presence of hydroxy (3546 cm^{-1}), carbonyl (1740 and 1715 cm^{-1}) and aromatic (1603 and 1451 cm^{-1}) functionalities. The ^1H and ^{13}C NMR resonances (Tables 1 and 2) of **1** were indicative of one benzoyloxy group [δ_H 7.95 × 2 (H-2′ and H-6′), 7.52 (H-4′), and 7.40 × 2 (H-3′ and H-5′); δ_C 167.1 (C-7′), 132.9 (C-4′), 130.6 (C-1′), 129.6 × 2 (C-2′ and C-6′) and 128.4 × 2 (C-3′ and C-5′)] and one acetate group [δ_H 2.17 (H-2″); δ_C 170.7 (C-1″) and 21.4 (C-2″)]. The ^1H NMR spectrum also contained signals for three olefinic protons [δ_H 5.58 (H-12), 5.35 (H-5) and 5.19 (H-11)], three oxygenated methine protons [δ_H 4.81 (H-14), 4.59 (H-3) and 3.99 (H-7)], three tertiary methyl groups [δ_H 1.61 (H_3-17), 1.14 (H_3-19) and 1.12 (H_3-18)] and two secondary methyl groups [δ_H 1.02 (H_3-16) and 0.97 (H_3-20)]. In addition to the resonances of one benzoyloxy group and one acetoxy group, the ^{13}C NMR spectra revealed twenty carbons, namely, four quaternary carbons, nine methines, two methylenes and five methyl carbons. Based on the co-occurring jatrophane diterpenes[15,17,19,20], the NMR data suggested that **1** was a jatrophane diester bearing one acetoxy group and one benzoyloxy group. Four structural fragments were established from the ^1H–^1H COSY spectrum of **1** (Fig. 2, blue thick lines). In the HMBC spectrum (Fig. 2), the correlations from H_2-1 to C-3, C-4, and C-15; from H-3 to C-1, C-5, C-15, and C-7′; from H-4 to C-1, C-2, C-6, C-14, and C-15; from H_3-16 to C-1, C-2, and C-3; and from OH-15 to C-1 and C-15 constructed a five-membered carbon ring that was fused to the macrocycle at the C-4 and C-15 positions and contained a methyl group (CH_3-16), a benzoyloxy group, and a hydroxyl group at the C-2, C-3, and C-15 positions, respectively. The HMBC correlations from H-5 to C-3, C-7, C-15, and C-17; from H_3-17 to C-5, C-6, and C-7; from H-7 to C-8 and C-9; from H_3-18 and H_3-19 to C-9, C-10, and C-11; from H-11 to C-9 and C-13; from H_3-20 to C-12, C-13, and C-14; and from H-14 to C-4, C-12, and C-1″ constructed a 12-membered macrocyclic skeleton, with four methyl groups (CH_3-17, CH_3-18, CH_3-19, and CH_3-20) at C-6, C-10 × 2, and C-13, respectively, a hydroxyl group at C-7, a keto-carbonyl at C-9, and an acetoxy group at C-14.

The NOESY correlations (Fig. 2) of H-3/H_3-16, H-3/H-4, H-4/H_3-17, H_3-17/H-11, and H-11/H-13 suggested that these protons were cofacial and were randomly assigned as α-oriented. On the other hand, the NOESY correlations of H-5/OH-7, H-5/OH-15, OH-15/H-12, H-12/H_3-20, and H_3-20/H-14 indicated that these were β-oriented. Moreover, the NOESY correlations observed for H-4/H_3-17, but not for H-5/H_3-17, together with the $^3J_{11,12}$ value of 15.5 Hz revealed that the Δ^5 and Δ^{11} double bonds were both in *E*-configurations. Fortunately, the absolute configuration of **1** could be determined by single-crystal X-ray crystallographic analysis and was found to be 2*R*, 3*S*, 4*S*, 7*R*, 13*R*, 14*R*, 15*R* (Fig. 3A) [Flack Parameter –0.08(14)] (Supplementary Information Table S1). Therefore, compound **1** was designated helioscopianoid A.

Compound **2** was isolated as colorless prisms and had a molecular formula of $C_{33}H_{44}O_9$ based on its (+)-HR-ESI-MS peak at *m/z* 607.2883 $[M + Na]^+$ (Calcd. for $C_{33}H_{44}O_9Na$, 607.2878). Detailed comparison of its 1D NMR data (Tables 1 and 2) showed compound **2** possessed the same parent skeleton as that of euphornin L[21]; the exception to this was the replacement of an acetoxy group with a hydroxyl group at the C-7 position in **2**. In addition, this deduction was supported by the upfield shift of H-7 from δ_H 4.85 to 3.80 and the diagnostic HMBC correlations from H-5 to C-7 and H_3-17 to C-7. The NOESY correlations observed for H-5/H-2′ and H-5/H-9 confirmed that the C-9 acetoxy group was α-oriented, and the β-orientation of the C-15 acetoxy group was established to be the same as that of **1** by the NOESY correlation of H-2′/H-2‴′ (Supplementary Information Fig. S15). Finally, based on X-ray diffraction analysis (Fig. 3B), the absolute configuration of **2** was determined to be 2*R*, 3*S*, 4*S*, 7*R*, 9*R*, 13*R*, 14*R*, 15*R* [Flack Parameter –0.01(8)] (Supplementary Information Table S2). The structure of **2** was therefore characterized and named helioscopianoid B.

Compound **3** was isolated as a white amorphous powder. Its molecular formula, $C_{33}H_{44}O_{10}$, was derived from its sodium adduct ion at *m/z* 623.2806 $[M + Na]^+$ (Calcd. 623.2827) of in its (+)-HR-ESI-MS data. The ^1H and ^{13}C NMR resonances (Tables 1 and 2) of compound **3** were characteristic of polyesterified jatrophanes and indicated **3** was structurally related to euphornin[15,22], differing only by the addition of a hydroxy group at C-2. This was supported by the observation of an oxygenated tertiary carbon resonance (δ_C 79.6) and the HMBC correlations from OH-2 (δ_H 4.05 in acetone-d_6) to C-1, C-2, and C-3 (Supplementary Information Fig. S29). The hydroxy group at C-2 was assigned as α-oriented by the NOESY correlations between OH-2 and H-3 and between H_3-16 and H-2′

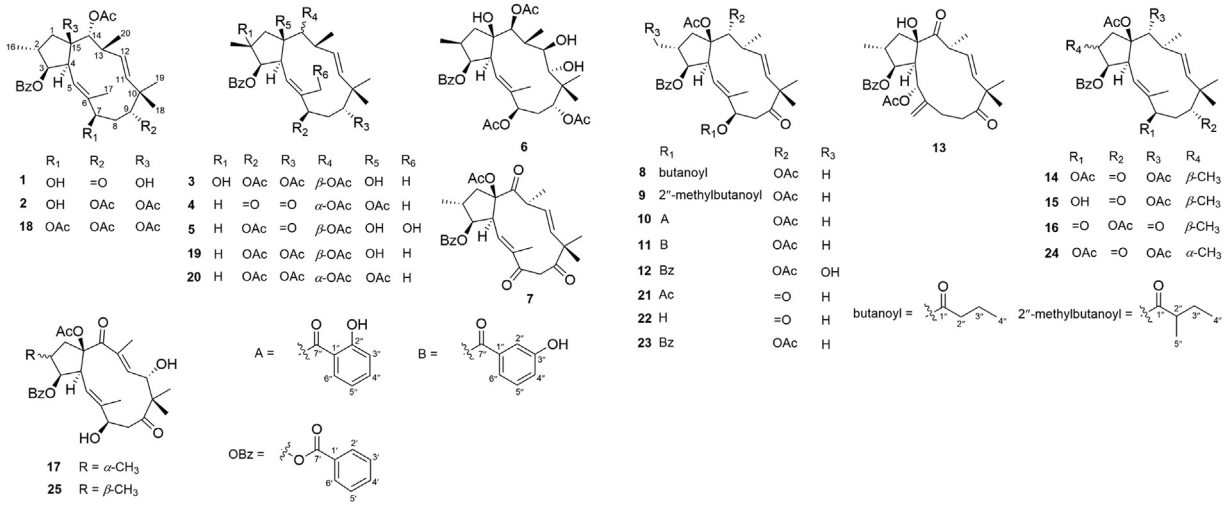

Figure 1 The structures of compounds **1**–**17**.

(Supplementary Information Fig. S30). In all the naturally occurring jatrophane diterpenoids reported to date[15,17,21], the five-membered ring and the macrocyclic skeleton are *trans*-fused, H-4 is α-oriented based on biosynthetic considerations, and the substituent at C-15 is β-oriented. The absolute configuration of **3** was elucidated as 2*R*, 3*R*, 4*S*, 7*R*, 9*R*, 13*R*, 14*S*, 15*R* based on the biogenetical *S*-configuration of C-4 and the absolute configurations of compounds **1** and **2**. Compound **3** was therefore named helioscopianoid C, and its structure was determined as shown.

Compound **4** was found to have a molecular formula of $C_{31}H_{38}O_8$ according to its HR-ESI-MS data (*m/z* 561.2469 $[M + Na]^+$, Calcd. for $C_{31}H_{38}O_8Na$, 561.2459). In contrast to the NMR data of co-occurring euphornin H[15,23], the ^{13}C NMR data of **4** (Table 2) revealed the presence of a second carbonyl group at δ_C 194.8 (C-7) and the absence of an oxygenated methine. The oxygenated methine in euphornin H had been replaced in **4** by a carbonyl group at C-7, which was confirmed by the HMBC correlations from H-5 (δ_H 6.99) and H_3-17 (δ_H 1.95) to C-7 (Supplementary Information Fig. S38). The stereocenters of **4** were assigned as 2*S*, 3*S*, 4*S*, 13*R*, 14*R*, 15*R*, which are the same as those of euphornin H. Thus, compound **4** (helioscopianoid D) was elucidated as shown.

Compound **5**, helioscopianoid E, was determined to have a formula of $C_{31}H_{40}O_9$ as deduced from its sodium adduct (+)-HR-ESI-MS ion peak at *m/z* 579.2551 $[M + Na]^+$ (Calcd. for $C_{31}H_{40}O_9Na$, 579.2565), indicating **5** contains one more oxygen atom than euphornin G[15]. A comparison of the overall NMR data of **5** (Tables 1 and 2, and Supplementary Information Figs. S43–54) and euphornin G showed they were structurally similar, except for the presence of a hydroxymethyl group in **5** (δ_C 61.7; δ_H 4.25 and 3.96). In addition, the HMBC correlations from H_2-17 to C-5, C-6, and C-7 indicated the hydroxy group was attached to C-17 (Supplementary Information Fig. S53). The relative configuration of **5** was found to be the same as that of euphornin G by comparing their NOESY correlations of H-2/H-4, H-3/H-4, H-4/H-13, H-4/H-14, H-4/H-17, and H-7/H-17 (Supplementary Information Fig. S54). The absolute configuration of **5** was elucidated as 2*S*, 3*S*, 4*S*, 7*R*, 13*R*, 14*S*, 15*R* based on the *S*-configuration of C-4 from our biosynthetic understanding and the absolute configurations of compounds **1** and **2**. The structure of **5** was therefore elucidated as shown.

The molecular formula of **6** was determined to be $C_{33}H_{46}O_{11}$ from its (+)-HR-ESI-MS data (*m/z* 641.2924 $[M + Na]^+$, Calcd. for $C_{33}H_{46}O_{11}Na$, 641.2932), and this formula corresponds to eleven indices of hydrogen deficiency. Compound **6** and euphornin[15,22] were structural analogues except two hydroxy groups at C-11 and C-12 (δ_C 76.0 and 66.4; δ_H 2.79 and 5.14) in **6** had replaced the corresponding olefinic protons of the 11,12-double bond in euphornin based on their 1D (Tables 1 and 2) and 2D (Supplementary Information Figs. S58–69) NMR. The OH-11 and OH-12 substituents of **6** were α-oriented and β-oriented, respectively, based on the NOESY correlations of H-11/H-5, H-11/H-9, H-11/H_3-18, H-12/H_3-19 and H-12/H_3-17 (Supplementary Information Fig. S69). The absolute configuration of the 11, 12-diol moiety was established using the $Mo_2(OAc)_4$-induced circular dichroism (ICD) method developed by Snatzke's and Frelek's group[24–26]. The positive Cotton effects observed at 317 nm in the ICD (Fig. 4) indicated the absolute configuration of **6** was 11*S* and 12*R*. The absolute configuration of **6** was confirmed to be 2*S*, 3*S* 4*S*, 7*R*, 9*R*, 11*S*, 12*R*, 13*R*, 14*S*, 15*R*. Therefore, the structure of helioscopianoid F (**6**) was characterized as shown.

Compound **7**, colorless crystals, was found to have a molecular formula of $C_{29}H_{34}O_7$ as indicated by its HR-ESI-MS (*m/z* 517.2204 $[M + Na]^+$, Calcd. 517.2197) and NMR data. From comparing the NMR spectra of **7** with those of euphoscopin E[15] (Supplementary Information Figs. S73–78), the differences between them suggested the presence of a carbonyl group (δ_C 195.6) at C-7 in **7** instead of the hydroxy group seen in euphoscopin E. This was confirmed by the HMBC correlations from H-5 and H_3-17 to C-7. Finally, the *E*-configurations of the $\Delta^{5,6}$ and $\Delta^{11,12}$ double bonds were determined from X-ray crystallographic data (Fig. 5), which also confirmed the absolute configuration of **7** to be 2*R*, 3*S*, 4*S*, 13*S*, and 15*R* [Flack Parameter 0.01(10)] (Supplementary Information Table S3). Compound **7**, helioscopianoid G, was thus structurally characterized.

Compound **8** showed a molecular formula of $C_{35}H_{46}O_9$ as deduced from its sodiated (+)-HR-ESI-MS ion at *m/z* 633.3046 $[M + Na]^+$ (Calcd. 633.3034). The ^1H and ^{13}C NMR spectra of **8** (Tables 2 and 3) were similar to those of **7** except for the presence of a butanoyl group [δ_C 173.1, 35.6 (1.49, 1H, m and 1.39, 1H, m),

Table 1 ^{1}H NMR spectroscopic data of compounds **1–6** (δ in ppm, J in Hz, $CDCl_3$).

Position	**1**[a]	**2**[a]	**3**[b]	**4**[c]	**5**[c]	**6**[b]
1α	1.76, dd (13.0, 6.0)	2.64 dd (14.5, 6.0)	2.08 s	2.54 dd (15.0, 7.8)	2.15[d]	2.36[d]
1β	1.23[d]	1.34 dd (14.5, 12.0)	2.08 s	2.01 dd (15.0, 8.4)	1.77 t (13.2)	1.63[d]
2	2.53 m	2.40 m		2.14 m	2.17 m	2.25 m
3	4.59 dd (9.0, 4.5)	4.74 t (9.5)	5.25 dd (5.2, 4.4)	5.59 t (3.6)	5.50 t (4.2)	5.42 t (3.6)
4	3.56 dd (11.5, 9.0)	3.68 dd (11.0, 9.5)	3.45 dd (10.8, 5.2)	3.33 dd (9.6, 3.6)	3.06 dd (9.6, 4.2)	2.73 dd (10.8, 3.6)
5	5.35 dq (11.5, 1.5)	5.71 dq (11.0, 1.5)	5.68 dq (10.8, 1.2)	6.99 dq (9.6, 1.2)	5.99 d (9.6)	6.01 dq (10.8, 1.2)
7	3.99 dd (7.5, 2.0)	3.80 dd (6.0, 4.5)	4.95 dd (6.0, 4.4)		5.20 dd (9.6, 3.0)	5.02 dd (6.0, 5.2)
8α	3.12 dd (15.0, 2.0)	2.00 dd (16.0, 4.5)	1.97 dd (14.8, 4.4)	4.03 d (13.2)	3.11 dd (13.8, 9.6)	1.91[d]
8β	2.40 dd (15.0, 7.5)	1.74 dd (16.0, 6.0)	1.93 dd (14.8, 6.0)	3.32 d (13.2)	2.59 dd (13.8, 3.0)	1.59[d]
9		4.55 dd (4.5, 2.5)	4.75 m			5.41 dd (6.0, 2.0)
11	5.19 d (15.5)	4.93 d (15.5)	5.09 d (15.6)	4.99 d (15.6)	4.96 d (15.6)	2.79 d (6.8)
12	5.58 dd (15.5, 10.0)	5.16 dd (15.5, 9.5)	5.62 dd (15.6, 9.2)	5.27 dd (15.6, 9.6)	5.70 dd (15.6, 8.4)	5.14 brs
13	2.36 m	2.31 m	2.62 m	2.13 m	2.65 m	1.66[d]
14	4.81 d (10.0)	6.04 d (10.0)	5.23 d (2.8)	5.98 d (10.2)	4.95 d (3.0)	4.91 d (1.6)
16	1.02 d (6.5)	1.06 d (6.5)	1.29 s	0.97 d (7.2)	0.96 d (7.2)	0.99 d (7.2)
17	1.61 d (1.5)	1.56 d (1.5)	1.76 d (1.2)	1.95 d (1.2)	4.25 brd (13.2) 3.96 brd (13.2)	1.68 d (1.2)
18	1.12 s	0.92 s	0.89 s	1.22 s	1.09 s	0.89 s
19	1.14 s	0.91 s	0.96 s	1.20 s	1.23 s	0.74 s
20	0.97 d (6.5)	0.93 d (6.5)	0.95 d (7.2)	0.92 d (7.2)	0.98 d (7.2)	1.02 d (7.2)
OBz-3						
2′,6′	7.95 dd (7.5, 1.5)	8.00 dd (8.0, 1.5)	8.06 dd (7.6, 1.2)	7.98 dd (7.8, 1.2)	8.03 dd (7.8, 1.2)	8.13 dd (7.6, 1.2)
3′,5′	7.40 t (7.5)	7.42 t (8.0)	7.44 t (7.6)	7.48 t (7.8)	7.45 t (7.8)	7.49 t (7.6)
4′	7.52 t (7.5)	7.53 t (8.0)	7.53 t (7.6)	7.60 t (7.8)	7.56 t (7.8)	7.57 t (7.6)
OAc-7			1.18 s		1.30 s	1.23 s
OAc-9		2.04 s	1.95 s			1.93 s
OAc-14	2.17 s	2.17 s	2.22 s	2.16 s	2.23 s	2.19 s
OAc-15		2.18 s		2.32 s		
OH-2			4.05 s[e]			
OH-7	4.61[d]					
OH-11						3.09 d (8.0)
OH-12						2.36[d]
OH-15	1.51 s		3.38 s[e]		3.94 s[e]	2.80 s

[a]500 MHz ^{1}H NMR.
[b]400 MHz ^{1}H NMR.
[c]600 MHz ^{1}H NMR.
[d]Overlapping signals.
[e]Data were measured in acetone-d_6.

18.1 (1.20, 2H, m), and 13.5 (0.60, 3H, t, J = 7.2 Hz)] at C-7 in **8** instead of the corresponding carbonyl group seen in **7**. These results were confirmed by the ^{1}H–^{1}H COSY and HMBC spectra, respectively (Supplementary Information Figs. S90 and S92). The butanoyl substituent was elucidated as β-oriented by the NOESY correlations of H-4/H_3-17/H-7 (Supplementary Information Fig. S93). Finally, on account of the biogenetical *S*-configuration of C-4 in jatrophane diterpenoids[15,17,18] and the absolute configuration of **7** that was established from X-ray crystallographic data, the absolute configuration of **8** was elucidated as 2*R*, 3*S*, 4*S*, 7*R*, 13*S*, 14*R*, 15*R*. Therefore, compound **8**, helioscopianoid H, can be represented as shown.

Compound **9** possessed a molecular formula of $C_{36}H_{48}O_9$ as established from its (+)-HR-ESI-MS data (*m/z* 647.3191 $[M+Na]^+$, Calcd. 647.3191). Its ^{1}H and ^{13}C NMR data (Tables 2 and 3) showed that the structure of **9** resembles that of compound **8**, except for a 2″-methylbutanoyl group at C-7 in **9** instead of the butanoyl group seen in **8**. In its ^{1}H–^{1}H COSY spectrum, the cross peaks of H-2″(H_3-5″)/H-3″/ H_3-4″ constructed the 2″-methylbutanoyl fragment (Supplementary Information Fig. S99). Moreover, in the HMBC spectrum of **9**, key correlations from H_3-5″ to C-1″, C-2″, and C-3″; from H_3-4″ to C-2″ and C-3″; and from H-7 to C-1″ further confirmed the structure and the position of 2″-methylbutanoyl group at C-7 in **9** (Supplementary Information Fig. S101). The absolute configuration of **9** was established as 2*R*, 3*S*, 4*S*, 7*R*, 13*S*, 14*R*, 15*R* based on it having the same absolute configuration as compounds **7** and **8**. Compound **9** was thereby named helioscopianoid I.

Compounds **10** and **11** were found to have the same molecular formula, $C_{38}H_{44}O_{10}$, based on their (+)-HR-ESI-MS and ^{13}C NMR data. Similar to related compounds **8** and **9**, the ^{1}H and ^{13}C NMR spectroscopic data of compounds **10** and **11** showed they possessed the same skeleton (Tables 3 and 4). The only differences between **10** and **11** were in the oxygenated substituents at C-7, and the differences were identified in their 2D NMR spectra. The ^{1}H–^{1}H COSY correlations of H-3″/H-4″/H-5″/H-6″ and the HMBC correlations of OH-2″/C-1″, C-2″, and C-3″; H-3″/C-1″ and C-5″; H-6″/C-2″, C-4″ and C-7″; and H-7/C-7″ established the structure of the 2″-hydroxybenzoyloxy moiety at C-7 of **10** (Supplementary Information Figs. S108 and S110). Meanwhile,

Table 2 ^{13}C NMR spectroscopic data of compounds **1–9** [δ (ppm), $CDCl_3$].

Position	**1**[a]	**2**[a]	**3**[b]	**4**[c]	**5**[c]	**6**[b]	**7**[b]	**8**[c]	**9**[b]
1	42.2	38.7	51.6	46.2	47.7	48.8	42.3	43.4	43.5
2	35.0	36.1	79.6	39.8	36.9	37.1	40.2	37.9	38.1
3	82.8	82.5	83.5	80.4	82.1	82.7	82.6	83.8	84.1
4	43.5	43.8	45.2	48.6	47.3	49.3	52.7	44.3	44.3
5	119.2	120.1	119.8	142.2	127.9	120.4	132.8	122.6	122.7
6	140.2	137.6	134.7	137.7	139.9	135.9	139.7	136.0	135.7
7	73.5	72.3	73.1	194.8	72.9	73.9	195.6	73.3	73.2
8	38.9	35.2	32.6	53.8	42.5	30.9	50.6	43.0	42.8
9	214.0	74.8	73.6	204.5	208.3	70.2	204.1	207.7	207.7
10	51.8	39.4	39.8	51.4	51.5	43.3	50.2	49.2	49.2
11	130.4	135.6	138.8	136.9	134.2	76.0	137.4	133.7	133.7
12	133.1	129.5	128.6	131.3	131.4	66.4	132.9	133.8	133.9
13	42.1	42.2	39.8	42.3	39.4	39.9	46.5	37.9	38.0
14	79.2	74.2	82.3	75.3	80.5	83.5	209.9	75.5	75.6
15	82.7	92.1	84.2	91.2	83.6	85.5	96.2	92.5	92.6
16	16.5	16.8	23.3	14.0	13.7	14.5	19.0	19.2	19.3
17	15.9	15.9	16.4	12.3	61.7	16.4	13.2	19.0	18.9
18	24.1	23.0	20.3	24.8	24.4	18.2	25.4	25.5	25.7
19	19.2	20.5	22.7	22.1	20.1	16.8	23.7	25.1	25.0
20	20.2	21.4	19.5	20.7	19.5	11.7	18.1	23.2	23.1
OBz-3									
1′	130.6	130.7	130.0	129.9	130.1	130.1	130.2	130.9	130.9
2′, 6′	129.6	129.5	129.9	129.6	129.9	129.9	129.5	129.6	129.6
3′, 5′	128.4	128.6	128.7	128.8	128.6	128.7	128.6	128.5	128.4
4′	132.9	133.1	133.2	133.5	133.2	133.2	133.3	132.9	132.9
7′	167.1	166.8	165.3	165.8	165.9	165.8	165.6	165.6	165.6
OR_1-7			Ac		Ac	Ac		Butanoyl	2′′-Methylbutanoyl
1′′			169.7		170.8	169.2		173.1	176.3
2′′			20.0		20.2	19.9		35.6	40.4
3′′								18.1	26.5
4′′								13.5	11.5
5′′									16.0
OAc-9		171.5	169.2			169.9			
		21.3	21.2			21.2			
OAc-14	170.7	170.0	171.7	169.8	170.8	170.6		170.2	170.2
	21.4	21.3	21.2	21.1	21.2	21.1		21.2	21.2
OAc-15		170.3		169.8			170.8	170.2	170.2
		23.0		22.4			22.1	22.3	22.2

[a]500 MHz ^1H NMR.
[b]400 MHz ^1H NMR.
[c]600 MHz ^1H NMR.

the ^1H–^1H COSY correlations of H-4′′/H-5′′/H-6′′ and the HMBC correlations of H-2′′/C-3′′, C-4′′, and C-6′′; H-4′′/C-2′′, C-3′′, and C-6′′; and H-6′′/C-2′′, C-4′′, and C-7′′ established the structure of the 3′′-hydroxybenzoyloxy moiety at C-7 of **11** (Supplementary Information Figs. S117 and S119). Compounds **10** and **11** were found to have the same configurations as **8** and **9** based on their NOESY spectra (Supplementary Information Figs. S111 and S120). The absolute configurations of **10** and **11** were both confirmed to be 2*R*, 3*S*, 4*S*, 7*R*, 13*S*, 14*R*, 15*R*, which are the same absolute configurations as seen in compounds **8** and **9**. Consequently, compound **10** (helioscopianoid J) and compound **11** (helioscopianoid K) were defined as depicted.

Compound **12** was isolated as a white powder. Analyses of its ^{13}C NMR and HR-ESI-MS data indicated a molecular formula of $C_{38}H_{44}O_{10}$ (*m/z* 683.2831 $[M + Na]^+$, Calcd. 683.2827), which is 16 mass units more than that of euphosopin C[17], suggesting the presence of an extra hydroxy group. The 1D NMR spectra of **12** (Tables 3 and 4) was similar to that of euphoscopin C except for the presence of an additional hydroxy group and the absence of a methyl group at C-16 in **12**. These findings suggested that the methyl group of euphoscopin C was replaced with a hydroxy group at C-16 in **12**, and this is supported by the diagnostic HMBC correlations of H_2-16/C-1, C-2, and C-3 (Supplementary Information Fig. S128). The NOESY correlations from H_2-16 to H-3 assigned the relative configuration of H_2-16 to be α-oriented (Supplementary Information Fig. S129). The absolute configuration of **12** was elucidated as 2*S*, 3*S*, 4*S*, 7*R*, 13*S*, 14*R*, 15*R* based on the biogenetical *S*-configuration of C-4 in jatrophane diterpenoids and the absolute configuration of compound **7**. Compound **12**, helioscopianoid L, was thus delineated as shown.

Compound **13** was obtained as a white powder. Its (+)-HR-ESI-MS indicated a molecular formula of $C_{29}H_{36}O_7$ based on the presence of a sodium ion peak at *m/z* 519.2376 $[M + Na]^+$ (Calcd. for $C_{29}H_{36}O_7Na$, 519.2353). Two ester residues were identified as one benzoyloxy group (δ_H 7.94, dd, J = 7.6, 1.6 Hz, 2H; 7.60, t, J = 7.6 Hz, 1H; and 7.49, t, J = 7.6Hz, 2H) and one acetoxy

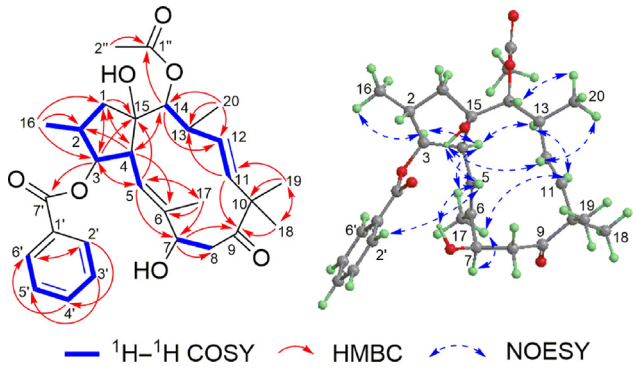

Figure 2 Key ^{1}H–^{1}H COSY, HMBC, and NOESY correlations for **1**.

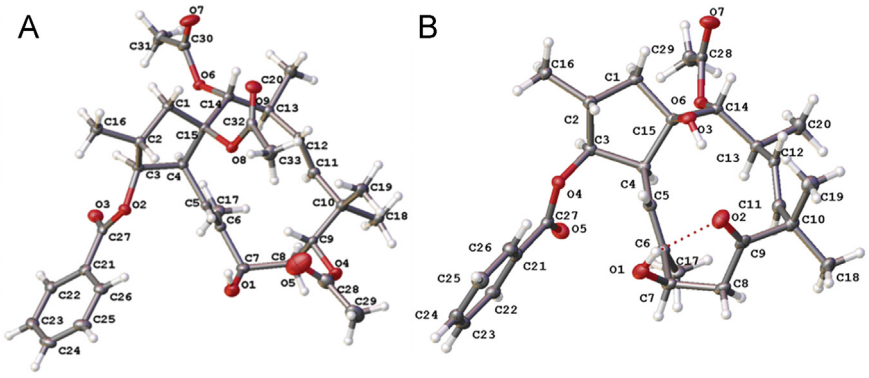

Figure 3 Single-crystal X-ray structures of **1** and **2**.

group (δ_C 170.2 and 21.1; δ_H 1.86, s, 3H) using the diagnostic ^{1}H NMR data (Tables 4 and 5). It was noted that the acetoxy group in **13** was an unusually far upfield (δ_H 1.86), possibly because of the shielding effect of the benzoyloxy group at C-3. Additionally, one *trans*-disubstituted double bond (δ_H 5.66, d, J = 16.0 Hz and 5.27, dd, J = 16.0, 9.2 Hz), one exocyclic double bond (δ_H 5.11, brs and 4.98, brs), one hydroxy group (δ_C 88.6), and two keto-carbonyls (δ_C 211.9 and 211.2) were distinguished from its ^{1}H and ^{13}C NMR spectra. From tis ^{1}H and ^{13}C NMR and HSQC spectra, **13** was found to have a polyesterified jatrophane-type diterpenoid core. The HMBC correlations of H-3/C-7′ and H-5/C-2″ placed the benzoyloxy group and the acetoxy group at C-3 and C-5, respectively (Supplementary Information Fig. S137). Two carbonyl groups and one hydroxy group were located at C-9, C-14 and C-15, respectively, by the HMBC correlations of H_3-18 and H_3-19/C-9 (δ_C 211.2), H_3-20/C-14 (δ_C 211.9), and OH-15/C-15 (δ_C 88.6). The HMBC correlations from H_3-18 to C-11 and from H_3-20 to C-12 indicated there was a double bond between C-11 and C-12, and the HMBC correlations from H_2-17 to C-5, C-6, and C-7 indicated a $\Delta^{6(17)}$ double bond. In the NOESY spectrum of **13** (Supplementary Information Fig. S138), the correlations of H-2′/H-5, H-5/H-7β, H-5/OH-15, H-7β/H-11, and H-11/H-13 indicated that these protons were cofacial, and they were randomly assigned as β-oriented. The NOESY correlations of H-4/H_3-16 revealed that these groups were α-oriented. In particular, the large value of $^3J_{4,5}$ (10.4 Hz) suggested an *endo*-type conformation of the $\Delta^{6(17)}$ double bond.[19] Hence, compound **13** was named helioscopianoid M.

Compound **14** had the same molecular formula, $C_{33}H_{42}O_9$ by (+)-HR-ESI-MS (*m/z* 605.2718 $[M + Na]^+$; Calcd. 505.2721), and the planar structure as that of euphoscopin B[21] according to its HR-ESI-MS and 1D (Tables 4 and 5) and 2D NMR data (Supplementary Information Figs. S144-147). However, the chemical shifts of C-1 (δ_C 48.0) and C-16 (δ_C 14.2) in **14** were very different from those of euphoscopin B[21], suggesting that the relative configuration of the methyl group at C-2 was not the same in the two compounds. In the NOESY spectrum of **14** (Supplementary Information Fig. S147), the correlation from H-2′ to H_3-16 suggested that H_3-16 was β-oriented as shown. That means compound **14** was the 2-*epi*-isomer of euphoscopin B, and this configuration is very common in jatrophanes isolated from *E. helioscopia*[15]. The absolute configuration of **14** was determined to be 2*S*, 3*S*, 4*S*, 7*R*, 13*S*, 14*R*, 15*R* based on the biogenetical *S*-configuration of C-4 and the absolute configurations of compounds **1** and **2**. Finally, the structure of **14**, helioscopianoid N, was established.

Compound **15** gave a quasimolecular ion peak at *m/z* 563.2629 $[M + Na]^+$ in its (+)-HR-ESI-MS data, which corresponds to a molecular formula $C_{31}H_{40}O_8$. Analysis of its HRMS and NMR data (Tables 4 and 5) suggested that compound **15** was closely related to **14**, except **15** contained one fewer acetoxy groups than **14**. Compared with the chemical shift of H-7 (δ_H 5.26) of **14**, the H-7 shift (δ_H 4.42) of **15** was significantly shielded ($\Delta\delta_H$ −0.84), indicating the presence of a hydroxy group at C-7 of **15**. This assignment was further confirmed by its HMBC spectrum (Supplementary Information Fig. S155). The absolute configuration of **15** was also determined to be 2*S*, 3*S*, 4*S*, 7*R*, 13*S*, 14*R*, 15*R*, which is the same as that of **14**. Thus, the structure of helioscopianoid O (**15**) was deduced as shown.

Compound **16** ($C_{31}H_{38}O_8$) was obtained as a white powder. Its ^{1}H and ^{13}C NMR spectra (Tables 4 and 5) indicated that **16** possessed the same carbon skeleton as **14** but showed some

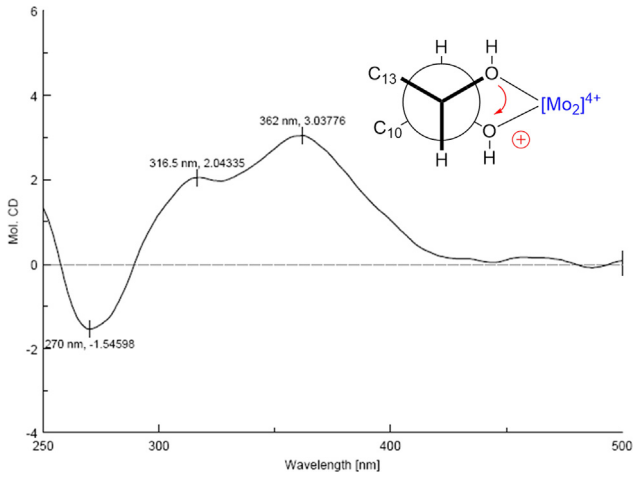

Figure 4 CD spectrum of **6** in DMSO containing $Mo_2(OAc)_4$ with the inherent CDs subtracted and preferred conformation of the 11,12-diol moiety in the chiral Mo complex of compound **6**.

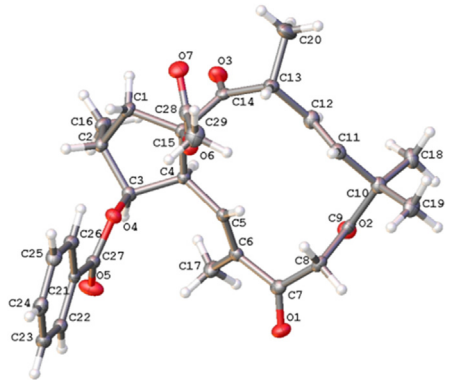

Figure 5 Single-crystal X-ray structure of **7**.

differences in the functional groups. Based on a comparison of its NMR spectra to those of **14**, the absence of an acetoxy group in **16** and the concomitant presence of an additional keto-carbonyl group suggested an acetoxy group had been replaced with a keto-carbonyl group in **16**. From its HMBC correlations of H_3-17 and H-5/C-7, H_3-20 and H-4/C-14, and H_3-18 and H_3-19/C-9 (Supplementary Information Fig. S164), two keto-carbonyl groups and one acetoxy group in **16** were located at C-7 (δ_C 199.2), C-14 (δ_C 212.1), and C-9 (δ_H 2.09; δ_C 170.5 and 21.3), respectively, which was a different substitution pattern than was seen in **14**. The α-orientation of OAc-9 was assigned from the NOESY correlations of H-5/H-11/H-9 (Supplementary Information Fig. S165). The absolute configuration of **16** was assigned as 2*S*, 3*S*, 4*S*, 7*R*, 13*S*, 15*R* on account of the biogenetical *S*-configuration of C-4 and the absolute configuration of compound **7**. The structure of helioscopianoid P (**16**) was therefore elucidated as shown.

Compound **17** was isolated as a white powder. Its molecular formula, $C_{29}H_{36}O_8$, was derived from its (+)-HR-ESI-MS data (*m/z* 535.2312 $[M + Na]^+$; Calcd. 535.2302) in conjunction with its ^{13}C NMR data. The planar structure of **17** was assigned based on its 1D NMR data (Tables 4 and 5) along with its ^1H–^1H COSY, HSQC, and HMBC spectra (Supplementary Information Figs. S171–173), and it was found to be identical to the scaffold of euphoheliosnoid D[27]. Notably, the downfield shift of C-16 from δ_C 13.8 to 18.3 and the upfield shifts of C-1 and C-2 from δ_C 47.1 and 39.4 to 43.3 and 38.6, respectively (Tables 3 and 5), suggested the relative configurations of the methyl group at C-2 was different in the two compounds. The NOESY correlations (Supplementary Information Fig. S174) of **17** between H-4 and H_3-16 indicated that H_3-16 was α-oriented as shown. Finally, the aforementioned data allowed the identification of **17** (helioscopianoid Q) as depicted.

Eight known diterpenoids, euphornin L (**18**)[21], euphornin (**19**)[15], euphornin D (**20**)[15], euphoscopin F (**21**)[17], euphoscopin E (**22**)[15], euphoscopin C (**23**)[17], euphoscopin B (**24**)[21], and euphoheliosnoid D (**25**)[27], were characterized by comparison of their spectroscopic data with the reported values. The 1D and 2D NMR spectra of euphornin D (**20**) and euphoscopin E (**22**) are also supplied in Supplementary Information Figs. S178–183 and Figs. S73–78, respectively.

The P-glycoprotein inhibitory activities of compounds **1**–**17** were evaluated by measuring the change in the intracellular adriamycin accumulation in the ADM-resistant human breast adenocarcinoma cell line (MCF-7) (Fig. 6)[23,28]. Cyclosporin A (CsA) was used as a positive control. Notably, compounds **8** and **16** at 20 μmol/L increased the accumulation of ADM approximately 3-fold compared to the negative control. The jatrophanes in *E. helioscopia* are mainly $\Delta^{5,6}\Delta^{11,12}$-jatrophane diterpenoids. The preliminary analysis of the structures of **1**–**17** and their corresponding P-gp inhibitory effects revealed that the presence of various acyl groups or carbonyl groups at C-7 in the same jatrophane core, especially the butanoyl group instead of hydroxy group at C-7, played an important role in the activity. The methyl group at C-2, in either the *R* or *S* configuration, as well as the methyl group at C-13, had no significant contribution to the inhibition of P-gp activity.

The neuroprotective activities of compounds **1**–**17** were examined based on two different models[29–32]. Compound **8** attenuated rotenone-induced PC12 cell damage by increasing the cell viability in the model from 61.4 ± 2.1% to 74.0 ± 4.7% at 10 μmol/L. Compounds **2**, **8**, and **12** exhibited activities against PC12 cells injured by serum deprivation, increasing the cell viability in the model from 56.5 ± 7.0% to 75.4 ± 4.6%, 74.2 ± 5.8%, and 72.3 ± 3.9% at 10 μmol/L, respectively (Table 6). The other compounds were inactive in the assays.

3. Conclusions

A total of 25 jatrophane-type diterpenoids, including 17 new jatrophane diterpenoid eaters (**1**–**17**) and eight known compounds (**18**–**25**), were isolated from the whole plants of *Euphorbia helioscopia*. The absolute configurations of compounds **1**, **2**, and **7** were accurately elucidated by X-ray crystallography. Among these new isolates, compounds **8** and **16** exhibited P-glycoprotein (ABCB1) inhibitory activities while compounds **2**, **8**, and **12** showed neuroprotective effects. To the best of our knowledge, this is the first report to evaluating the neuroprotective effects of jatrophane diterpenoids. As components of *Euphorbia helioscopia*, more detailed chemical and biological investigations of the plant metabolites are required to determine their contributions to support and enhance the application of the herbal medicine and to search for hits for new drug development.

Table 3 ^{1}H NMR spectroscopic data of compounds **7–12** (δ in ppm, J in Hz, $CDCl_3$).

Position	**7**[a]	**8**[b]	**9**[a]	**10**[b]	**11**[b]	**12**[b]
1α	2.80 dd (14.4, 7.6)	2.97 dd (15.6, 8.4)	2.99 dd (15.2, 8.4)	2.99 dd (15.0, 7.8)	3.02 dd (15.6, 8.4)	2.96 dd (15.6, 9.0)
1β	2.18 dd (14.4, 9.6)	1.44 dd (15.6, 9.0)	1.45 dd (15.2, 9.2)	1.50 dd (15.0, 7.8)	1.50 dd (15.6, 7.8)	1.51 dd (15.6, 10.2)
2	2.29 m	2.16 m	2.16 m	2.15 m	2.14 m	2.22 m
3	5.34 dd (7.2, 2.8)	5.16 dd (7.2, 2.4)	5.14 dd (7.2, 2.4)	5.17 dd (7.2, 2.4)	5.17 dd (7.2, 2.4)	5.24 dd (7.2, 2.4)
4	3.15 t (7.2)	3.25 dd (9.0, 7.2)	3.24 dd (8.4, 7.2)	3.30 dd (8.4, 7.2)	3.29 dd (9.0, 7.2)	3.24 dd (9.0, 7.2)
5	6.56 dq (7.2, 1.2)	5.69 dq (9.0, 1.2)	5.71 dq (8.4, 1.2)	5.85 dq (8.4, 1.2)	5.83 dq (9.0, 1.2)	5.90 dq (9.0, 1.2)
7		5.42 dd (11.4, 4.2)	5.47 dd (11.6, 4.4)	5.69 dd (11.4, 4.2)	5.66 dd (11.4, 4.2)	5.75 dd (11.4, 4.2)
8α	4.39 d (14.8)	3.15 dd (15.6, 11.4)	3.16 dd (16.0, 11.6)	3.34 dd (16.2, 11.4)	3.31 dd (16.2, 11.4)	3.38 dd (16.2, 11.4)
8β	3.18 d (14.8)	2.67 dd (15.6, 4.2)	2.65 dd (16.0, 4.4)	2.82 dd (16.2, 4.2)	2.84 dd (16.2, 4.2)	2.86 dd (16.2, 4.2)
11	5.68 d (15.6)	5.35 d (16.2)	5.36 d (16.4)	5.39 d (16.2)	5.37 d (16.2)	5.41 d (16.2)
12	5.07 dd (15.6, 9.6)	5.17 dd (16.2, 9.0)	5.16 dd (16.4, 8.8)	5.21 dd (16.2, 9.0)	5.19 dd (16.2, 9.0)	5.22 (16.2, 9.0)
13	3.51 m	2.44 m	2.44 m	2.46 m	2.47 m	2.48 m
14		5.91 d (1.8)	5.92 d (1.6)	5.95 d (1.8)	5.93 d (1.8)	5.91 d (1.8)
16	1.28 d (7.2)	1.10 d (7.2)	1.10 d (7.2)	1.08 d (7.2)	1.09 d (7.2)	3.71 brd (10.8)3.51 dd (10.8, 3.0)
17	1.74 d (1.2)	1.86 d (1.2)	1.85 d (1.2)	1.95 d (1.2)	1.94 d (1.2)	1.95 d (1.2)
18	1.31 s	1.10 s	1.10 s	1.31 s	1.14 s	1.14 s
19	1.16 s	1.27 s	1.28 s	1.14 s	1.31 s	1.34 s
20	1.14 d (7.2)	0.92 d (7.2)	0.91 d (7.2)	0.94 d (7.2)	0.92 d (7.2)	0.94 d (7.2)
OBz-3						
2′,6′	7.95 dd (7.6, 1.6)	7.99 dd (7.8, 1.8)	7.99 dd (8.0, 1.6)	7.84 dd (7.8, 1.2)	7.87 dd (7.8, 1.2)	7.92 dd (7.8, 1.2)
3′,5′	7.44 t (7.6)	7.43 t (7.8)	7.43 t (8.0)	7.27 t (7.8)	7.31 t (7.8)	7.34 t (7.8)
4′	7.57 t (7.6)	7.55 t (7.8)	7.55 t (8.0)	7.42 t (7.8)	7.47 t (7.8)	7.50 t (7.8)
OR_1-7		Butanoyl	2′′-Methylbutanoyl	A	B	Bz
2′′		1.49 m, 1.39 m	1.48 m		7.02 dd (3.0, 1.8)	7.52 dd (7.8, 1.2)
3′′		1.20 m	1.27 m, 1.05 m	6.79 dd (7.8, 1.2)		6.89 t (7.8)
4′′		0.60 t (7.2)	0.61 t (7.2)	7.22 td (7.8, 1.2)	6.84 ddd (7.8, 3.0, 1.8)	7.27 t (7.8)
5′′			0.64 d (7.2)	6.20 td (7.8, 1.2)	6.90 t (7.8)	6.89 t (7.8)
6′′				7.14 dd (7.8, 1.2)	7.18 dt (7.8, 1.8)	7.52 dd (7.8, 1.2)
OAc-14		2.15 s	2.15 s	2.17 s	2.17 s	2.15 s
OAc-15	2.34 s	2.22 s	2.22 s	2.22 s	2.20 s	2.18 s
OH-2′′				10.6 s		

[a]400 MHz ^{1}H NMR.
[b]600 MHz ^{1}H NMR.

4. Experimental

4.1. General experimental procedures

Melting points were measured on an X-6 analyzer. Optical rotations were measured on a JASCO P-2000 automatic digital polarimeter. UV spectra were recorded on a JASCO V-650 spectrometer. CD spectra were measured on a JASCO-815 CD spectrometer. IR spectra were measured on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission). NMR spectra were obtained at 400, 500, or 600 MHz for ^{1}H NMR and 100, 125, or 150 MHz for ^{13}C NMR on Varian Mercury-400, Bruker AVANCE III 500 MHz or Bruker AVIIIHD 600 MHz spectrometers, in $CDCl_3$ or acetone-d_6, and solvent peaks were used as references. HR-ESI-MS data were acquired using an Agilent 6520 Accurate-Mass Q-TOF LC/MS spectrometer. The X-ray crystallographic data were collected on an Agilent Xcalibur Eos Gemini diffractometer with graphite monochromated Cu-K_α radiation (λ = 1.5418 Å). Analytical HPLC separations were conducted on an Agilent 1260 infinity system equipped with a DAD-UV. Preparative HPLC separations were performed on a CXTH system (Beijing Chuangxintongheng instrument Co. Ltd., China), equipped with a UV3000 detector using a YMC-Pack ODS-A column (250 mm × 20 mm, 5 μm, Kyoto, Japan). Polyamide (30–60 mesh, Changzhou Changfeng Chemical Factory, China), silica gel (100–200, 200–300 mesh, Qingdao Marin Chemical Inc. Qingdao, China), CHP20P MCI gel (75–150 μm, Mitsubishi Chemical Corporation), Sephadex LH-20 (GE), and ODS-A-HG (50 μm, YMC, Japan) were used for column chromatography (CC).

4.2. Plant material

The whole plants of *E. helioscopia* L. were purchased from Anguo Materia Medica Market in Hebei Province, China, in September 2015 and were identified by Professor Lin Ma, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (ID-S-2624) has been deposited at the Herbarium of the Department of Medicinal Plants, the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.

4.3. Extraction and isolation

The dried and powdered whole plants of *Euphorbia helioscopia* (55 kg) were exhaustively extracted with 80% EtOH (3 × 140 L)

Table 4 ^{13}C NMR spectroscopic data of compounds **10–17** [δ (ppm), $CDCl_3$].

Position	**10**[b]	**11**[b]	**12**[b]	**13**[a]	**14**[b]	**15**[b]	**16**[b]	**17**[a]
1	43.7	43.8	37.1	50.7	48.0	47.8	44.4	43.3
2	38.1	38.0	46.6	38.0	37.6	37.5	38.6	38.6
3	84.0	84.6	81.5	84.8	81.1	81.8	79.8	84.0
4	44.2	44.3	45.5	49.2	45.7	46.1	54.5	49.2
5	123.4	122.8	122.4	70.0	122.9	121.3	134.6	118.4
6	135.1	135.6	135.8	145.1	137.1	140.3	144.0	141.1
7	74.9	74.6	74.3	30.0	73.6	72.1	199.2	73.7
8	42.9	42.7	42.7	35.3	44.0	46.0	37.8	37.9
9	207.5	207.7	207.5	211.2	208.0	210.2	77.3	227.6
10	49.3	49.3	49.3	50.0	49.2	49.3	41.2	51.3
11	133.8	133.6	133.5	137.4	134.7	135.1	138.8	77.3
12	133.9	133.9	134.2	131.0	133.0	131.9	128.7	138.1
13	37.9	38.3	37.7	43.7	39.2	39.2	45.8	135.9
14	75.7	75.8	75.4	211.9	77.2	77.4	212.1	200.5
15	92.4	92.6	92.6	88.6	90.3	90.1	95.5	95.2
16	19.3	19.5	64.4	18.9	14.2	14.3	15.0	18.3
17	19.0	18.9	18.9	115.4	19.4	18.9	13.5	15.7
18	25.5	25.8	25.8	24.3	25.8	26.3	29.2	24.0
19	25.1	25.0	24.9	24.0	24.6	24.2	18.4	23.0
20	23.0	22.8	23.2	17.6	22.4	22.3	19.3	12.7
OBz-3								
1′	130.4	130.5	130.1	130.0	130.6	130.3	130.0	130.6
2′,6′	129.3	129.4	129.6	129.6	129.6	129.5	129.7	129.7
3′,5′	128.3	128.4	128.5	128.8	128.4	128.6	128.6	128.5
4′	132.9	133.0	133.3	133.5	132.9	133.3	133.4	133.1
7′	169.6	166.0	166.5	165.8	165.4	166.6	165.8	166.2
OR_1-7	A	B	Bz		Ac			
1″	112.3	131.4	129.9		170.0, 20.2			
2″	161.6	115.9	129.3					
3″	117.5	155.5	128.1					
4″	135.3	119.9	132.6					
5″	118.8	129.5	128.1					
6″	129.6	121.7	129.3					
7″	169.6	165.8	166.1					
OAc-5				170.2, 21.1				
OAc-9							170.5, 21.3	
OAc-14	170.1, 21.2	170.2, 21.2	170.3, 21.1		170.1, 21.1	170.1, 21.1		
OAc-15	170.2, 22.2	170.2, 22.2	170.3, 22.2		169.7, 22.1	169.9, 22.1	170.8, 21.9	170.7, 21.9

[a]400 MHz ^1H NMR.
[b]600 MHz ^1H NMR.

under reflux. The EtOH was evaporated *in vacuo*, and the crude extract (8.5 kg) was diluted with H_2O and successively partitioned with petroleum ether, EtOAc, and *n*-BuOH (three times with 20 L each). To remove most of the chlorophylls and flavones, the EtOAc extract fraction (1.2 kg) was treated with polyamide in water, 50% EtOH and 95% EtOH sequentially. After removing the solvent, the 50% EtOH eluate (350 g) was chromatographed on silica gel eluting with a petroleum ether–acetone gradient system (30:1 to 1:1) to afford 18 fractions (A1–A18) based on TLC analysis. Fr. A9 (4.7 g) was applied to MPLC on an ODS-A-Hg column and eluted with MeOH/H_2O (50:50 to 100:0) to obtain 27 subfractions (A9-1–A9-27). After further purification by Sephadex LH-20 CC (MeOH), A9-23 (102 mg), A9-24 (201 mg), and A9-25 (98 mg) were submitted to preparative HPLC (75% MeCN in H_2O, 10 mL/min) and afforded **8** (3.2 mg), **9** (4.2 mg), and **10** (2.8 mg), respectively. Fr. A9-22 (2.1 g) was subjected to Sephadex LH-20 (MeOH) and preparative HPLC (70% MeCN) and yielded compounds **18** (146 mg), **21** (834 mg), and **23** (312 mg). Fr. A11 (14.1 g) was fractionated by MCI gel CC, eluted with MeOH/H_2O (70:30 to 90:10) and produced four subfractions (A11-m1–A11-m4). A11-m2 (8.6 g) was subjected to MPLC on an ODS-A-HG column (MeOH/H_2O, 70:30 to 100:0) to afford 12 fractions (A11-m2-1–A11-m2-12). Preparative HPLC was used to isolate compounds **1** (28.0 mg), **2** (43.3 mg), **13** (4.2 mg), **14** (3.0 mg), and **15** (2.4 mg) from A11-m2-1 (65% MeCN), A11-m2–2 (65% MeCN), A11-m2-3 (65% MeCN), A11-m2-5 (70% MeCN), and A11-m2-10 (70% MeCN), respectively. After purification by Sephadex LH-20 (MeOH) and preparative HPLC (70% MeCN), Fr. A11-m2-6 gave compounds **19** (2.7 g) and **24** (1.8 g). A11-m3 (2.8 g) was subjected to CC over silica gel eluting with a gradient of petroleum ether (15:1 to 2:1) in acetone and gave five subfractions, A11-m3-1–A11-m3-5. A11-m3-2 (207 mg) was purified by Sephadex LH-20 (MeOH) and preparative HPLC (70% MeCN) to afford **4** (2.0 mg), **7** (60.7 mg), and **20** (15.2 mg), and A11-m3-3 (370 mg) was also separated to yield **16** (4.2 mg) and **22** (151 mg) using the same procedure. Fr. A17 (20.0 g) was separated on a column of silica gel eluted with $CHCl_3$–MeOH (100:1 to

Table 5 ^{1}H NMR spectroscopic data of compounds **13–17** (δ in ppm, J in Hz, $CDCl_3$).

Position	**13**[a]	**14**[b]	**15**[b]	**16**[b]	**17**[c]
1α	2.25 dd (14.4, 8.4)	2.61 dd (15.6, 8.4)	2.63 dd (15.6, 8.4)	3.14 dd (15.6, 10.2)	2.64 dd (13.6, 6.4)
1β	1.78 dd (14.4, 3.2)	2.21 dd (15.6, 12.0)	2.28 dd (15.6, 12.0)	1.89 dd (15.6, 10.2)	2.45 dd (13.6, 7.6)
2	2.41 m	1.96 m	1.99 m	2.62 m	2.42 m
3	5.43 brd (4.4)	5.59 t (4.2)	5.55 t (4.2)	5.73 t (4.8)	5.13 dd (6.4, 3.2)
4	3.49 dd (10.4, 4.4)	3.28 dd (8.4, 4.2)	3.30 dd (8.4, 4.2)	2.88 dd (9.0, 4.8)	3.15 dd (10.8, 6.4)
5	5.44 brd (10.4)	5.70 dq (8.4, 1.2)	5.75 dq (8.4, 1.2)	6.74 dq (9.0, 1.2)	5.97 dq (10.8, 1.2)
7α	2.64 m	5.26 dd (11.4, 4.8)	4.42 dd (10.2, 4.8)		4.15 t (7.6)
7β	2.20 m				
8α	2.55 m	3.05 dd (15.0, 11.4)	2.88 dd (15.0, 10.2)	2.82 dd (13.8, 3.6)	3.25 dd (14.8, 2.8)
8β	2.42 m	2.70 dd (15.0, 4.8)	2.76 dd (15.0, 4.8)	2.73 dd (13.8, 7.8)	2.39[d]
9				4.98 dd (7.8, 3.6)	
11	5.66 d (16.0)	5.36 d (16.2)	5.34 d (16.2)	5.31 d (15.6,)	4.02 t (10.0)
12	5.27 dd (16.0, 9.2)	5.14 dd (16.2, 9.0)	5.11 dd (16.2, 9.0)	5.04 dd (15.6, 9.0)	6.41 dd (10.0, 1.6)
13	4.05 m	2.38 m	2.34 m	3.31 m	
14		6.07 d (1.8)	6.07 d (1.8)		
16	1.14 d (7.2)	0.90 d (7.2)	0.96 d (7.2)	1.01 d (7.2)	1.22 d (6.8)
17	5.11 brs, 4.98 brs	1.87 d (1.2)	1.83 d (1.2)	1.80 d (1.2)	1.51 d (1.2)
18	1.19 s	1.20 s	1.17 s	0.90 s	1.29 s
19	1.25 s	1.09 s	1.09 s	0.91 s	1.07 s
20	1.17 d (7.2)	0.91 d (7.2)	0.90 d (7.2)	1.10 d (7.2)	1.78 d (1.6)
OBz-3					
2′,6′	7.94 dd (7.6, 1.6)	7.97 dd (7.8, 1.2)	7.95 dd (7.8, 1.2)	8.02 dd (7.8, 1.2)	8.06 dd (7.6, 1.6)
3′,5′	7.49 t (7.6)	7.42 t (7.8)	7.42 t (7.8)	7.45 t (7.8)	7.47 t (7.6)
4′	7.60 t (7.6)	7.54 t (7.8)	7.55 t (7.8)	7.59 t (7.8)	7.58 t (7.6)
OAc-5	1.86 s				
OAc-7		1.31 s			
OAc-9				2.09 s	
OAc-14		2.16 s	2.17 s		
OAc-15		2.25 s	2.13 s	2.33 s	2.14 s
15-OH	2.59 s				

[a]400 MHz.
[b]600 MHz.
[c]Overlapping signals.

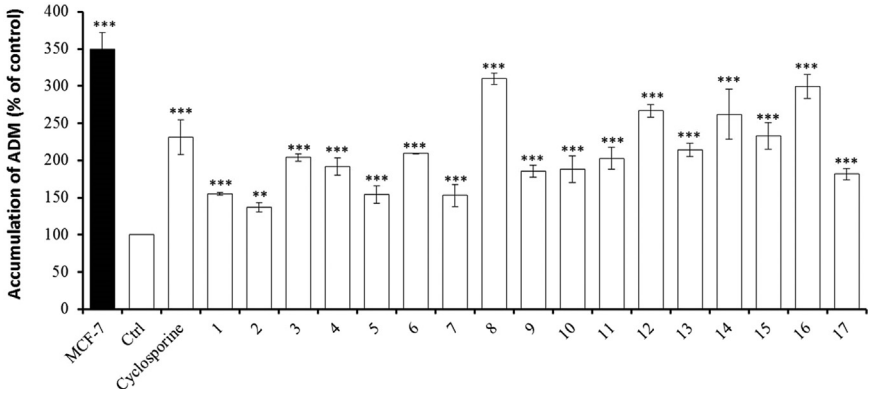

Figure 6 Effects of compounds **1–17** on the accumulation of ADM in an ADM-resistant human breast adenocarcinoma cell line (MCF-7). Cyclosporine was used as a positive control. All of the compounds were tested at 20 μmol/L. ($^*P<0.05$ *vs*. control, $^{**}P<0.01$ *vs* control, $^{***}P<0.001$ *vs*. control).

15:1) to give nine fractions (A17-1–A17-9). A17-7 (7.5 g) was subjected to MPLC on an ODS-A-HG column (MeOH/H_2O, 60:40 to 100:0) and yielded 25 subfractions, A17-7-1–A17-7-25. Among these subfractions, A17-7-8 (680 mg) was further purified by Sephadex LH-20 (MeOH) and preparative HPLC (65% MeCN) to afford **3** (13.5 mg), **11** (4.8 mg), and **12** (3.2 mg). A17-7-9 (600 mg) was also passed over Sephadex LH-20 (MeOH), and then further purified by preparative HPLC (60% MeCN), to yield **5** (3.0 mg), **6** (13.2 mg), **17** (3.4 mg), and **25** (14.3 mg).

Table 6 Neuroprotective effects of compounds **2**, **8**, and **12** on the survival rate of PC12 cells injured by serum deprivation (10 μmol/L mean ± SD, $n = 9$)[a].

Group	Survival rate (% of control)
Control	100.0 ± 4.4
Model	56.5 ± 7.0
NGF[b]	76.2 ± 5.2***
2	75.4 ± 4.6***
8	74.2 ± 5.8***
12	72.3 ± 3.9***

[a]###$P<0.001$ *vs.* control, ***$P<0.001$ *vs.* model, **$P<0.01$ *vs.* model, *$P<0.1$ *vs.* model.
[b]Positive control substance.

4.3.1. *Helioscopianoid A (**1**)*

Colorless crystals (MeOH); mp 147–148 °C; $[\alpha]_D^{20}$ +24.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (logε) 227 (4.04) nm; IR ν_{max} 3546, 3499, 2960, 1740, 1715, 1603, 1451, 1370, 1231, 1028, 918, 706 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; (+)-HR-ESI-MS *m/z* 521.2528 $[M + Na]^+$ (Calcd. for $C_{29}H_{38}O_7Na$, 521.2510).

4.3.2. *Helioscopianoid B (**2**)*

Colorless crystals (MeOH); mp 155–156 °C; $[\alpha]_D^{20}$ −103.8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (logε) 229 (3.84) nm; IR ν_{max} 3444, 2969, 1747, 1716, 1602, 1452, 1373, 1230, 1026, 977, 715 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; (+)-HR-ESI-MS *m/z* 607.2883 $[M + Na]^+$ (Calcd. for $C_{33}H_{44}O_9Na$, 607.2878).

4.3.3. *Helioscopianoid C (**3**)*

White, amorphous; $[\alpha]_D^{20}$ −45.9 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (logε) 229 (3.94) nm; IR ν_{max} 3536, 3436, 2969, 1732, 1715, 1600, 1452, 1368, 1278, 1050, 907, 718 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; (+)-HR-ESI-MS *m/z* 623.2806 $[M + Na]^+$ (Calcd. for $C_{33}H_{44}O_{10}Na$, 521.2510).

4.3.4. *Helioscopianoid D (**4**)*

White, amorphous; $[\alpha]_D^{20}$ +93.6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (logε) 228 (4.10) nm; IR ν_{max} 2972, 1746, 1721, 1603, 1453, 1371, 1240, 1027, 982, 714 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; (+)-HR-ESI-MS *m/z* 561.2469 $[M + Na]^+$ (Calcd. for $C_{31}H_{38}O_8Na$, 561.2459).

4.3.5. *Helioscopianoid E (**5**)*

White, amorphous; $[\alpha]_D^{20}$ +55.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (logε) 227 (4.22) nm; IR ν_{max} 3505, 2970, 1723, 1603, 1452, 1371, 1275, 1240, 1025, 919, 714 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; (+)-HR-ESI-MS *m/z* 579.2551 $[M + Na]^+$ (Calcd. for $C_{31}H_{40}O_9Na$, 579.2565).

4.3.6. *Helioscopianoid F (**6**)*

White, amorphous; $[\alpha]_D^{20}$ +70.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (logε) 229 (4.00) nm; IR ν_{max} 3515, 2973, 1739, 1715, 1602, 1452, 1372, 1275, 1044, 945, 715 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; (+)-HR-ESI-MS *m/z* 641.2924 $[M + Na]^+$ (Calcd. for $C_{33}H_{46}O_{11}Na$, 641.2932).

4.3.7. *Helioscopianoid G (**7**)*

Colorless crystals (MeOH); mp 189–190 °C; $[\alpha]_D^{20}$ +33.9 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (logε) 226 (4.15) nm; IR ν_{max} 2978, 1706, 1601, 1453, 1376, 1274, 1237, 1045, 976, 720 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 2 and 3; (+)-HR-ESI-MS *m/z* 517.2204 $[M + Na]^+$ (Calcd. for $C_{29}H_{34}O_7Na$, 517.2197).

4.3.8. *Helioscopianoid H (**8**)*

White, amorphous; $[\alpha]_D^{20}$ +32.1 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (logε) 228 (3.99) nm; IR ν_{max} 2968, 1738, 1715, 1602, 1453, 1370, 1221, 1020, 964, 714 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 2 and 3; (+)-HR-ESI-MS *m/z* 633.3046 $[M + Na]^+$ (Calcd. for $C_{35}H_{46}O_9Na$, 633.3034).

4.3.9. *Helioscopianoid I (**9**)*

White, amorphous; $[\alpha]_D^{20}$ +38.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (logε) 228 (4.23) nm; IR ν_{max} 2970, 1731, 1602, 1453, 1370, 1221, 1020, 964, 714 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 2 and 3; (+)-HR-ESI-MS *m/z* 647.3191 $[M + Na]^+$ (Calcd. for $C_{36}H_{48}O_9Na$, 647.3191).

*4.3.9.1. Helioscopianoid G (**10**).* White, amorphous; $[\alpha]_D^{20}$ −13.5 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (logε) 227 (4.16) nm; IR ν_{max} 3417, 2967, 1745, 1714, 1601, 1454, 1371, 1279, 1020, 989, 757, 712 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 3 and 4; (+)-HR-ESI-MS *m/z* 683.2840 $[M + Na]^+$ (Calcd. for $C_{38}H_{44}O_{10}Na$, 683.2827).

*4.3.9.2. Helioscopianoid K (**11**).* White, amorphous; $[\alpha]_D^{20}$ −97.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (logε) 229 (4.08) nm; IR ν_{max} 3423, 2969, 1716, 1603, 1453, 1370, 1219, 1021, 962, 706 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 3 and 4; (+)-HR-ESI-MS *m/z* 683.2848 $[M + Na]^+$ (Calcd. for $C_{38}H_{44}O_{10}Na$, 683.2827).

*4.3.9.3. Helioscopianoid L (**12**).* White, amorphous; $[\alpha]_D^{20}$ +19.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (logε) 228 (4.08) nm; IR ν_{max} 3490, 2969, 1716, 1602, 1451, 1314, 1276, 1026, 963, 713 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 3 and 4; (+)-HR-ESI-MS *m/z* 683.2831 $[M + Na]^+$ (Calcd. for $C_{38}H_{44}O_{10}Na$, 683.2827).

*4.3.9.4. Helioscopianoid M (**13**).* White, amorphous; $[\alpha]_D^{20}$ −12.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (logε) 226 (3.97) nm; IR ν_{max} 3494, 2971, 1715, 1602, 1452, 1371, 1277, 1029, 977, 714 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 4 and 5; (+)-HR-ESI-MS *m/z* 519.2353 $[M + Na]^+$ (Calcd. for $C_{29}H_{36}O_7Na$, 519.2353).

*4.3.9.5. Helioscopianoid N (**14**).* White, amorphous; $[\alpha]_D^{20}$ +27.8 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (logε) 227 (3.84) nm; IR ν_{max} 2966, 1741, 1717, 1602, 1452, 1368, 1242, 1024, 919, 713 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 4 and 5; (+)-HR-ESI-MS *m/z* 605.2718 $[M + Na]^+$ (Calcd. for $C_{33}H_{42}O_9Na$, 605.2721).

*4.3.9.6. Helioscopianoid O (**15**).* White, amorphous; $[\alpha]_D^{20}$ +97.8 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (logε) 227 (3.85) nm; IR ν_{max} 3552, 2971, 1745, 1705, 1603, 1451, 1367, 1218, 1020, 992, 717 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 4 and 5; (+)-HR-ESI-MS *m/z* 563.2629 $[M + Na]^+$ (Calcd. for $C_{31}H_{40}O_8Na$, 563.2615).

*4.3.9.7. Helioscopianoid P (**16**).* White, amorphous; $[\alpha]_D^{20}$ −95.6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (logε) 229 (4.06) nm; IR ν_{max} 2968, 1736, 1602, 1453, 1370, 1248, 1027, 947, 715 cm^{-1}; ^{1}H and

^{13}C NMR ($CDCl_3$), see Tables 4 and 5; (+)-HR-ESI-MS *m/z* 561.2470 $[M + Na]^+$ (Calcd. for $C_{31}H_{38}O_8Na$, 561.2459).

*4.3.9.8. Helioscopianoid Q (**17**).* White, amorphous; $[\alpha]_D^{20}$ +44.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (logε) 230 (4.30) nm; IR ν_{max} 3482, 2968, 1718, 1602, 1452, 1371, 1275, 1028, 932, 714 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 4 and 5; (+)-HR-ESI-MS *m/z* 535.2312 $[M + Na]^+$ (Calcd. for $C_{29}H_{36}O_8Na$, 535.2302).

4.4. Crystal structure analysis

Colorless crystals of **1**, **2**, and **7** were obtained from MeOH. Crystal data were collected on an Xcalibur Eos Gemini diffractometer with graphite monochromated Cu-K_α radiation (λ = 1.5418 Å). The crystal structures of **1**, **2**, and **7** were elucidated by direct methods using the SHELXS-97 program and refined by the full-matrix least-squares difference Fourier method. Crystallographic data of **1**, **2**, and **7** have been deposited at The Cambridge Crystallographic Data Center with the deposition numbers CCDC 1562748 for **1**, CCDC 1562749 for **2**, and CCDC 1562750 for **7** and are available free of charge *via* the Internet at www.ccdc.cam.uk/products/csd/request.

4.5. Determination of the absolute configuration of the 11,12-diol moieties in compound 6 by Snatzke and Frelek's method

For compound **6**, a 1/1.2 mixture of diol/$Mo_2(OAc)_4$ was subjected to ECD measurements at a concentration of 1.0 mg/mL according to the published procedure[24,33]. The first ECD spectrum was recorded immediately after mixing at room temperature, and the evolution of the curve over time was monitored until the curve remained constant (approximately 10 min after mixing). The inherent ECD spectrum was subtracted. The absolute configuration was elucidated by the diagnostic band at approximately 310–340 nm in the induced ECD spectrum.

4.6. MDR reversal assays[28]

Human breast adenocarcinoma cells (MCF-7) and adriamycin-resistant MCF-7 cells were seeded in a 24-well plate at a density of 1×10^5 cells per well and incubated for 48 h. After preincubation with fresh medium containing either cyclosporin A (20 μmol/L) or one of the test compounds (20 μmol/L) for 10 min, 10 μmol/L adriamycin was added to the medium. The plates were incubated for 1 h at 37 °C with gentle shaking. The reaction was terminated by removal of the medium. Cells were then washed three times with 1 mL of ice-cold PBS. The cell monolayers were subsequently lysed with 0.3 mL of 0.1% Triton X-100, and the concentration of adriamycin in the cell lysate was determined by LC–MS/MS. Protein concentrations served as the loading control and were measured using the bicinchoninic acid procedure with bovine serum albumin as the standard (Solarbio, China). MCF-7 cells with maximum adriamycin accumulation were used to compare with adriamycin-resistant MCF-7 cells to illustrate that this model was successfully established.

4.7. PC12 cell protection assay

The potential neuroprotective effects of compounds **1**–**17** against serum deprivation-induced and rotenone-induced PC12 cell damage were assayed with the MTT method in *vitro*. PC12 cells at a density of 5×10^3 cells per well in 96-well plates were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum and 5% horse serum and incubated at 37 °C in a humidified, 5% CO_2 atmosphere for 24 h. Then, the cells were cultured with or without test compounds (10 μmol/L) in DMEM without serum. After incubation for 48 h, 10 μL of MTT (5 mg/mL) was added and maintained for another 4 h. Absorbance was measured at 550 nm using an Ultramark microplate reader. The survival rate of PC12 cells was evaluated. Nerve Growth Fator (NGF, 0.05 μg/mL) was used as a positive control for the model of PC12 cells injured by serum deprivation. In another model, PC12 cells were seeded in 96-well plates at a density of 5×10^3 cells per well and incubated for 48 h. Then, compounds (10 μmol/L) and/or rotenone (4 μmol/L) were added to the cells. After incubation for another 48 h, 10 μL of MTT (5 mg/mL) was added and maintained for 4 h. Absorbance was measured at 570 nm using an Ultramark microplate reader. Cell viability was evaluated.

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Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.apsb.2018.03.011.

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