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Iridoids from *Valeriana jatamansi* induce autophagy-associated cell death *via* the PDK1/Akt/mTOR pathway in HCT116 human colorectal carcinoma cells

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ABSTRACT

Chlorovaltrates U-W (**1–3**), three previously undescribed iridoids, together with four known analogues were isolated from the roots of *Valeriana jatamansi*. Their structures were elucidated by means of spectroscopic analyses (HRESIMS, NMR). The cytotoxicity of all isolates was evaluated. Compounds **5–7** exhibited selective cytotoxicity against HCT116 cells, with IC_{50} values of 9.3, 1.7 and 2.2 μM, respectively. The preliminary mechanistic study revealed that, the cytotoxicity effect of **6** was attributed to Akt/mTOR activation blockade *via* inhibition of PDK1 phosphorylation. Meanwhile, compound **6** could induce autophagosome formation in HCT116 cells *via* suppressing its downstream Akt/mTOR. These findings show that compound **6** could be of great importance to the development of anti-colon cancer agents.

1. Introduction

Colorectal cancer (CRC) is one of the most frequent fatal cancers in the worldwide, with more than 600,000 deaths each year [1,2]. The current standard treatment of surgery, radiation and new chemotherapeutic has decreased mortality significantly, but recurrence and drug resistance frequently have occured within a short time [3,4]. Recently, patients with CRC have been progressively improving with novel agents targeting EGFR, such as cetuximab [5]. However, therapeutics targeting this signaling pathway is only effective for a subset of patients [6]. Since the occurrence and development of CRC results from the genetic variations in a number of oncogenes and tumor suppressor genes, it is ultimately critical to develop novel inhibitors for other targets. PDK1 (pyruvate dehydrogenase kinase-1) is a Ser/Thr kinase and globular protein constituted by two main domains of C-terminal PH domain and N-terminal kinase domain. PDK1-mediated phosphorylation is a key event in Akt/mTOR signal activation and it has been investigated in detail in later years. PDK1/Akt/mTOR signaling pathway plays an important role in the cellular survival, proliferation, and metabolism [7,8]. Recently, Xu et al. observed increased expression of PDK1 in colorectal cancer clinical specimens and cell lines and explored the key roles of PDK1 in CRC cells. High expression of PDK1 is associated with poor postoperative prognosis, drug resistance and higher recurrence rates [9,10]. Meanwhile, PDK1 inhibitors have been reported to exhibit strong anti-tumor effects or sensitize rapamycin in colorectal cancer cells [11]. Thus, the strategy of targeting PDK1 would provide greater therapeutic benefits to other anticancer agents.

Natural products play an important part in the discovery and development of drug-lead compounds [12–14]. *Valeriana jatamansi* Jones is an annual herb widely distributed in China and India [15]. Its roots have been extensively used as a traditional medicine for the treatment of diseases, such as tranquilizing hypnotic, nervous disorders, epilepsy, insanity, snake poisoning, skin diseases, and anxiety [15–18]. Previous chemical investigation on *V. jatamansi* revealed the presence of iridoids, sesquiterpenoids, essential oil, flavones and lignans [16,19–26]. Valepotriates, a type of iridoid with a variety of biological significance, have been attracting great interest in natural products. Phytochemical studies on *Valeriana wallichii* and *V. jatamansi* have resulted in a series of valepotriates, and some of which showed potent antitumor activities [19,27–33]. What is worth mentioning, volvaltrate B induced significant percentage of definitive remissions of ovarian tumors in the female mice [30]. To date, our

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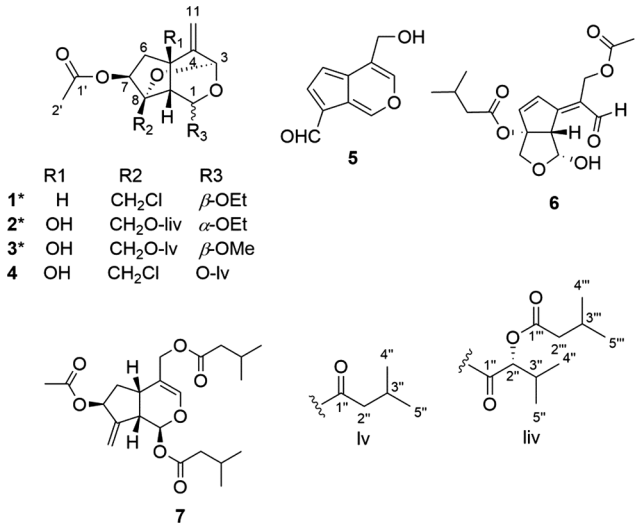

Fig. 1. Structures of compounds **1–7**.

results have revealed the presence of 3, 8-epoxy iridoids, chlorovaltrates P-T, as constituents of *V. jatamansi*. and weak cytotoxicity of these compounds against the lung adenocarcinoma cells A 549 and gastric carcinoma cells SGC7901 [34]. Although previous studies have been focused on anti-tumor activities, the targeted colon cancer and mechanism research of the iridoids derived from *V. jatamansi* have not been well studied.

In the present study, repeated purification *via* column chromatography of the EtOAc-soluble extract of *V. jatamansi* resulted in the isolation of three undescribed iridoids named chlorovaltrates U-W (**1–3**) and four known analogues (**4–7**) (Fig. 1). Herein, the isolation, structural elucidation and cytotoxicity effect of these compounds and the mechanism of targeted against colorectal carcinoma HCT116 cells are described.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on an Anton Paar MCP 200 (Anton Paar, Graz, Austria) at room temperature. UV spectra were recorded on a Shimadzu UV-260 spectrophotometer (Shimadzu, Kyoto, Japan). HRESIMS were acquired on a Waters Synapt G_2HDMS (Waters, Millford, MA, USA). The 1D and 2D NMR spectra were carried out on a Bruker-AVII-400 spectrometer (Bruker, Bremerhaven, Germany) with tetramethylsilane as internal standard. Column chromatography and preparative TLC were performed with silica gel (200–300 and 300–400 mesh, Qingdao Marine Chemical Co., Ltd., China), Sephadex LH-20 (Pharmacia, Sweden), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Japan). TLC was performed on a precoated GF254 plate (Qingdao Marine Chemical Co., Ltd., China). Spots were visualized under UV light followed by heating after spraying with 10% H_2SO_4 in EtOH solution. MPLC was performed on a MPLC-52 system (Saipuruisi, Beijing, China). Semipreparative HPLC was performed on Shimadazu LC-10AT instrument with an SPD-10AVP detector and a YMC-Pack ODS-A column (250 mm × 10 mm, 5 μm) at 210 nm. The human colorectal carcinoma cell lines HCT116, human lung cancer cell lines A549, human bone osteosarcoma epithelial cell lines U20S, human diffuse large B lymphoma cell lines OCI-ly10, human acute myeloid leukemia cell lines THP-1, were obtained from American Type Culture Collection (Manassas, VA, USA).

2.2. Plant material

The roots of *Valeriana jatamansi* Jones were collected from the medicinal herb market in Chengdu, Sichuan Province, China, in June 2015, and authenticated by Prof. Min Li. A voucher specimen (No. ZZX-1506) was deposited at the laboratory of Chinese medicinal chemistry, Chengdu University of TCM.

2.3. Extraction and isolation

The dried roots of *V. jatamansi* (4 kg) were extracted with 75% EtOH (3 × 30 L), to afford a crude extract (650 g) after removal of the solvent in *vacuo*. The crude extract was suspended in H_2O (2 L) and successively partitioned with petroleum ether, EtOAc and *n*-BuOH. The EtOAc layer (100 g) was subjected to a silica gel column using $CHCl_3$-MeOH (100:0 → 0:100, v/v) as eluent to afford six fractions (Fr. A-F). Fraction D (18 g) was further separated into six subfractions (Fr. D.1-D.6) on a silica gel column (petroleum ether-EtOAc, 100:0 → 20:80, v/v). Fraction D.4 (1 g) was subjected to MPLC with a gradient of MeOH-H_2O (50:50 → 100:0, v/v) as eluent, to give six subfractions (Fr.D.4.1-D.4.6). Fraction D.4.2 (150 mg) was applied to a Sephadex LH-20 column ($CHCl_3$-MeOH, 40:60, v/v), followed by preparative HPLC using CH_3CN-H_2O (75:25, v/v) as the mobile phase, to afford **2** (3.2 mg), **4** (5.8 mg), and **5** (2.7 mg). Fraction D.4.3 (200 mg) was chromatographed over Sephadex LH-20 ($CHCl_3$-MeOH, 40:60, v/v) to give four subfractions (Fr.D.4.3.1-D.4.3.4). Fraction D.4.3.2 (28 mg) was purified by preparative TLC (petroleum ether/EtOAc, 10:1) to yield **1** (3.5 mg). Fraction D.4.3.4 (30 mg) was purified by preparative HPLC (MeOH/H_2O, 70:30), then followed by Sephadex LH-20 (MeOH) to yield compounds **3** (2.5 mg). Compounds **6** (3.2 mg) and **7** (2.1 mg) were obtained from fraction D.4.4 by preparative HPLC (CH_3CN-H_2O, 75:25, v/v).

2.4. Chlorovaltrate U (1)

Colorless oil; $[\alpha]_D^{20}$: +65.96 (*c* 0.10, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} (log ε) 231 (3.25) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 325.0818 $[M + Na]^+$ (calcd for $C_{14}H_{19}ClO_5Na$, 325.0819).

2.5. Chlorovaltrate V (2)

Colorless oil; $[\alpha]_D^{20}$: −36.06 (*c* 0.16, CH_2Cl_2); UV(MeOH) λ_{max} (log ε) 232 (3.87) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 507.2208 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_{10}Na$, 507.2206).

2.6. Chlorovaltrate W (3)

Colorless oil; $[\alpha]_D^{20}$: +2.76 (*c* 0.11, CH_2Cl_2); UV(MeOH) λ_{max} (log ε) 236 (3.48) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 393.1524 $[M + Na]^+$ (calcd for $C_{18}H_{26}O_8Na$ 393.1525).

Table 1
^{1}H NMR (400 MHz) data of compounds **1–3** in CDCl3.

No.	1	2	3
1	5.09, d (3.1)	5.19, d (3.2)	5.06, d (0.7)
3	5.14, s	5.21, s	5.21, s
5	3.26, m		
6a	1.88, ddd (14.5, 7.6, 2.8)	2.00, dd (14.4, 2.8)	1.94, dd (14.1, 3.4)
6b	2.25, dd (14.5, 7.3)	2.56, dd (14.4, 7.3)	2.65, dd (14.1, 7.4)
7	5.09, m	4.91, dd (7.3, 2.8)	4.90, dd (7.4, 3.4)
9	2.52, dd (5.0, 3.1)	2.52, d (3.2)	2.38, brs
10a	3.78, d (11.5)	4.29, d (11.5)	4.46, d (12.0)
10b	3.82, d (11.5)	4.43, d (11.5)	4.62, d (12.0)
11a	4.84, d (0.9)	5.05, s	5.14, s
11b	4.93, d (0.9)	5.32, s	5.29, s
2′	2.06, s	2.05, s	1.99, s
OH		3.28, s	
OMe			3.47, s
OEt	3.49, m; 3.80, m	3.49, m; 3.80, m	
	1.17, t (7.1)	1.18, t (7.1)	

The signals of the substituent at C-10 in ^{1}H NMR.

For **2** (the α-isovaleroxyisovaleroxy group): δ_H 4.78 (1H, d, J = 4.6 Hz, H-2″), 2.20 (1H, m, H-3″), 1.01 (6H, d, J = 6.9 Hz, H-4″ and H-5″), 2.28 (2H, m, H-2‴), 2.12 (1H, m, H-3‴), 0.98 (6H, J = 6.9 Hz, H-4‴ and H-5‴); For **3** (the α-isovaleroxy group): δ_H 2.18 (2H, m, H-2″), 2.08 (1H, m, H-3″), 0.93 (3H, d, J = 6.6 Hz, H-4″), 0.93 (3H, d, J = 6.6 Hz, H-5″).

Table 2
^{13}C NMR (100 MHz) data of compounds **1–3** in $CDCl_3$.

No.	1	2	3
1	94.5	95.6	96.4
3	93.7	94.1	93.9
4	148.2	152.5	151.4
5	37.1	77.4	77.4
6	40.6	46.8	47.3
7	78.2	74.6	74.1
8	82.2	81.9	82.5
9	42.9	45.7	47.9
10	46.0	65.0	66.0
11	108.1	107.9	108.2
1′	169.7	169.9	169.6
2′	21.2	21.2	21.1
OMe			56.3
OEt	63.4, 15.3	63.6, 15.2	

In ^{13}C NMR. For **2** (the α-isovaleroxyisovaleroxy group): δ_C 169.7 (C, C-1″), 76.7 (CH, C-2″), 30.1 (CH, C-3″), 17.4 (CH_3, C-4″), 18.9 (CH_3, C-5″), 172.8 (C, C-1‴), 43.2 (CH_2, C-2‴), 25.8 (CH, C-3‴), 22.5 (CH_3, C-4‴, 5‴); For **3** (the α-isovaleroxy group): δ_C 172.8 (C, C-1″), 43.3 (CH_2, C-2″), 25.7 (CH, C-3″), 22.5 (CH_3, C-4″), 22.5 (CH_3, C-5″)

2.7. Cytotoxicity assay

The cytotoxic activity was measured by MTT assay. Five cancer cells including A549, U20S, OCI-ly10, THP-1 and HCT-116, were obtained from American Type Culture Collection (Manassas, VA, USA) and grown in RPMI-1640 culture medium containing 10% fetal bovine serum in 5% CO_2 at 37 °C. During the logarithmic growth phase, the cells were harvested and seeded in 96-well plates (2000–5000 cells/well) and treated 24 h later with various concentrations of compounds **1–7**. Paclitaxel (> 99%, Must Co., LTD, China) ranging from 0.003 μM to 10 μM was used as positive control. After 72 h of incubation, 20 μL/well of 5 mg/ml MTT was added to all wells and incubated for another 2–4 h. Finally, the formazan crystals were dissolved with acidified SDS (20%, w/v) overnight, and cell viability was measured by observing absorbance at 492 nm on a Varioskan flash-3001.

2.8. Autophagy detection

Autophagy was determined by analyzing the formation of LC3 puncta of autophagosomes in cells treated by compound **6**. Briefly, HCT116 cells were cultured in a confocal dish and transfected with mRFPGFP-LC3 adenovirus (Ad-tf-LC3) (Hanbio Co., LTD, China) before receiving treatment. Next, HCT116 cells transfected with mRFPGFP-LC3 adenovirus were treated by compound 6 for 20 h. Finally, image acquisition was performed using a confocal microscope (OLYMPUS, Japan). Cells with five or more mRFP-GFP-LC3 dots were recorded as Autophagic cell.

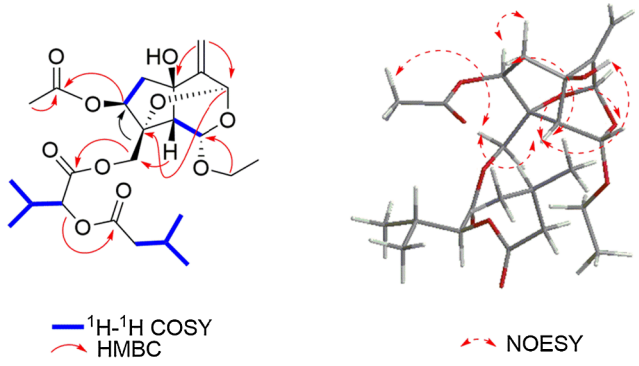

Fig. 2. Selected COSY, HMBC and NOESY correlations of compound **2**.

2.9. Western blot analysis

After treatment with a series of concentrations of compound **6** for 20 h at 37 °C, HCT116 cells were harvested, and the total proteins were extracted with RIPA buffer (10 mM Tris-HCl (pH 7.8), 1% NP40, 0.15 M NaCl, 1 mM EDTA, 10 μM aprotinin, 1 mM NaF and 1 mM Na_3VO_4). Protein concentrations were determined using BCA Protein Assay Kit. For western blot assay, equal proteins were separated by SDS-PAGE and blotted onto PVDF membranes and probed with various primary antibodies (1:1000) Including phospho-PDK1, PDK1, phospho-Akt, Akt, phospho-mTOR, mTOR, LC3-II, β-actin. Finally, Blots were developed with horseradish peroxidase (HRP)-conjugated secondary antibodies and Chemiluminescence Kit on Kodak X-ray films.

3. Results and discussion

3.1. Phytochemical investigation

Chlorovaltrate U (**1**) was obtained as colorless oil. The molecular formula was determined to be $C_{14}H_{19}ClO_5$ on the basis of the HRESIMS $[M + Na]^+$ at m/z 325.0818 (calcd for $C_{14}H_{19}ClO_5Na$, 325.0819), indicating five indices of hydrogen deficiency. The ^{1}H NMR data of **1** revealed an ethoxyl group [δ_H 3.49 (1H, m), 3.80 (1H, m), 1.17 (3H, t, J = 7.1 Hz)], an acetoxyl group [δ_H 2.06 (3H, s)], three methylenes [δ_H 1.88 (1H, ddd, J = 14.5, 7.6, 2.8 Hz), 2.25 (1H, dd, J = 14.5, 7.3 Hz), 3.78 (1H, d, J = 11.5 Hz), 3.82 (1H, d, J = 11.5 Hz), 4.84 (1H, d, J = 0.9 Hz), 4.93 (1H, d, J = 0.9 Hz)]. The NMR data of **1** were similar to those of 3, 8-epoxy-1-*O*-ethyl-5-hydroxyvalechlorine [22] except for C-5 and the adjacent carbons, indicating the absence of a hydroxyl group at C-5 in **1**, which was further supported by the HRESIMS. The ethoxyl and acetoxyl group were attached to C-1 and C-7, which was confirmed by the HMBC correlations from methylene proton of ethyoxyl to C-1 and from H-7 to C-1′. Further analysis of the 2D NMR data led to the assignment of all the proton and carbon signals for **1** (Tables 1 and 2). The relative configuration of **1** was established by the NOESY experiment. The NOESY correlations of H-5 with H-6β and H-6β with H-9 indicated that these protons were cofacial. NOESY correlations of H-10 with H-2′, H-9 with H-10, and no NOESY correlations of H-5 with H-7 and H-1 with H-5, indicated that acetoxyl at C-7 and CH_2Cl at C-8 had β-orientation, and H-1 was α-oriented. The result was in accord with naturally occurring iridoids exhibiting an α-orientation for H-1 and β-orientation for H-9 [24,31,35]. Therefore, the structure of **1** was elucidated and named as Chlorovaltrate U.

Chlorovaltrate V (**2**) possessed the molecular formula of $C_{24}H_{36}O_{10}$ based on the HRESIMS at m/z 507.2208 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_{10}Na$, 507.2206) with 7 degrees of unsaturation. The 1D NMR spectrum in combination with HSQC exhibited 24 carbon signals, including one ethoxyl group [δ_H 3.49 (1H, m), 3.80 (1H, m), 1.18 (3H, t, J = 7.1 Hz)], an acetoxyl group [2.05 (3H, s)], an α-isovaleroxyisovaleroxy moiety [δ_H 4.78 (1H, d, J = 4.6 Hz), 2.20 (1H, m), 1.01 (6H, d, J = 6.9 Hz), 2.28 (2H, m), 2.12 (1H, m), 0.98 (6H, d, J = 6.9 Hz)]. Its ^{1}H and ^{13}C NMR data were analogous to those of chlorovaltrate S [34], suggesting that they shared a similar structure. The only difference between **2** and chlorovaltrate S was the methoxyl at C-1 in chlorovaltrate S was replaced by an ethoxy group in **2**. Additionally, the HMBC correlations from methylene proton of ethyoxyl to C-1, from H-7 to C-1′ and from H-10 to C-1″ revealed an ethoxyl located at C-1, an acetoxyl at C-7 and an α-[(isovaleryloxy)isovaleryloxy] at C-

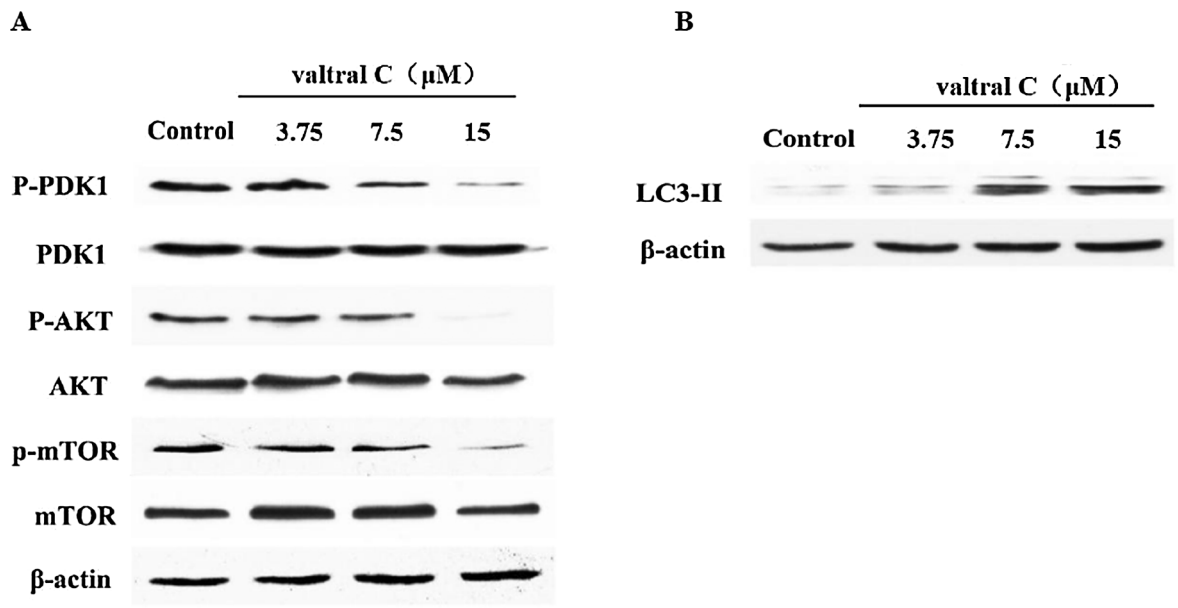

Fig. 3. Valtral C inhibited Akt/mTOR signal and regulated the expressions of autophagy related protein. A, After 20 h treatment with valtral C, the phosphorylation statuses of PDK1, Akt and mTOR in HCT116 cells were detected by immunoblot. B, After 20 h treatment of valtral C, a dose-dependent increase in the level of LC3-II was observed in HCT116 cells.

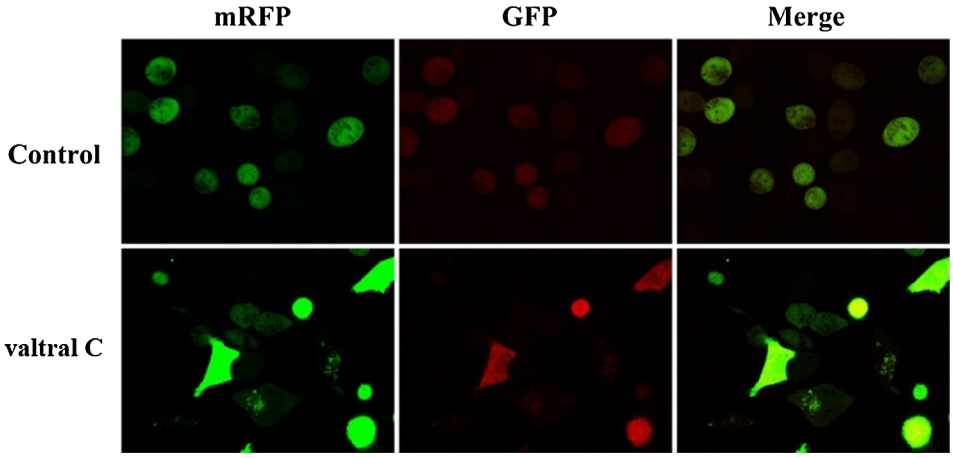

Fig. 4. Valtral C induced autophagy in HCT116 cells. After 20 h valtral C treatment, HCT116 cells transfected with mRFP-GFP-LC3 adenovirus were observed under a confocal fluorescence microscopy. Images are presented to indicate the cellular localization patterns of the mRFP-GFP-LC3 fusion protein.

10, respectively. By further analysis of the 2D NMR spectra, all the proton and carbon signals for **2** were unambiguously assigned (Tables 1 and 2). As shown in Fig. 2, the NOESY correlations of H-9 with H-1 but not with methylene proton of ethyoxyl suggested β-configuration for H-1. Moreover, the NOESY cross peaks of H-9 with HO-5, H-9 with H-10, and H-2′ with H-10 indicated β-configuration for the acetoxy at C-7. Consequently, compound **2** was defined and named as Chlorovaltrate V.

Chlorovaltrate W (**3**) was obtained as colorless oil. Its molecular formula was determined as $C_{18}H_{26}O_8$ by the HRESIMS $[M + Na]^+$ at *m/z* 393.1524 (calcd for $C_{18}H_{26}O_8Na$ 393.1525). The 1D NMR spectrum in combination with the HSQC showed one methoxyl [δ_H 3.47 (3H, s)], an acetoxyl group [δ_H 1.99 (3H, s)] and one α-isovaleroxy group [δ_H 2.18 (2H, m), 2.08 (1H, m), 0.93 (6H, d, J = 6.6 Hz)]. The NMR data of **3** were similar to those of compound **2**, suggesting that they were structural analogues, except for the presence of one methoxyl and α-isovaleroxy in **3**. The relative configuration of **3** was established by NOESY experiment. NOESY correlations of H-9 with MeO-1/H-10/H-6β, H-2′ with H-10, and no NOESY correlation between H-7 and H-9, indicate that methoxyl, acetoxyl and α-isovaleroxy were all β-oriented. Thus, the structure of **3** was elucidated and named as Chlorovaltrate W.

In addition to three novel iridoids **1–3**, the known compounds were identified by comparison of spectroscopic data with those reported in the literature as chlorovaltrate A (**4**) [31], desacylbaldrinal (**5**) [36], valtral C (**6**) [37] and desoxidodidrovaltrate (**7**) [38].

3.2. Compound 6 exhibits cytotoxicity effect via inhibiting Akt/mTOR signaling pathway in HCT116 cells

Activation of PI3K/Akt/mTOR signaling pathway is closely associated with the occurrence, development and metastasis of colon cancer [7,8]. The inhibitors of PI3K/Akt/mTOR signaling have been designed as a potential therapeutic target [39,40]. By using the MTT method, compounds **1–7** were tested in *vitro* against several human cancer cell lines including lung adenocarcinoma cells (A549), human osteosarcoma cells (U20S), diffuse large B-cell lymphoma cells (OCI-ly10), acute myelo-monocytic leukemia cells (THP-1) and human colorectal cancer cells (HCT-116). As shown in Table S1, Compounds **5–7** exhibited selective cytotoxicity against HCT116 cell with IC_{50} values of 9.3, 1.7 and 2.2 μM, respectively. In the previous study, the compound **6**, a decomposition product of valepotriates from *Valeriana jatamansi*, showed

selective cytotoxicity against metastatic prostate cancer (PC-3 M) and colon cancer (HCT-8) cell lines [37]. The cytotoxic activity of the compound **6** was in accordance with the previous result, but the mechanism of the component targeted colon cancer remained unknown. Fortunately, compound **6** was sufficient to meet our demand for further research. To elucidate the mechanism of anti-colon carcinoma activity, we detected the ability of compound **6** to inhibit the critical signaling pathways in HCT116 cells. After a 20 h treatment with gradient concentrations of compound **6**, HCT116 cells were harvested and lysed for a western blot assay. As shown in Fig. 3, compound **6** significantly inhibited the phosphorylation of PDK1 at higher concentration of 15 μM in a dose-dependent manner. The Phosphorylation of the downstream signaling proteins Akt/mTOR was also strongly inhibited by compound **6** consistent with the inhibition of PDK1 in a dose-dependent manner. Meanwhile, compound **6** did not modulate the expression of these proteins at the corresponding concentration. These results indicated that inhibiting the activation of PDK1 and its downstream target Akt/mTOR contribute to the *cytotoxicity* effect.

3.3. Compound 6 induces autophagosome formation in HCT116 cells

Recently, autophagy has been considered to be another way for cells as a novel "programmed" cell death [41–43]. It is well established that the Akt/mTOR signal plays a critical role in autophagy suppression. Inhibition of the activity of Akt or its downstream target mTOR contribute to trigger the onset of autophagy. Since compound **6** could inhibit Akt/mTOR by inhibiting PDK1, we detected its potential for inducing autophagy. The fluorescence formation of mRFP-GFP-LC3 puncta is well-characterized method to indicate autophagosomes. Compound **6** can trigger massive flow of autophagy *via* inducing the formation of large number of autophagy-lysosome (yellow) and autophagy body (green) in HCT116 cells (Fig. 4). LC3-BII is the membrane bound form of LC that is usually used as an autophagy marker. Consistent with the formation of mRFP-GFP-LC3 puncta, western blot analysis demonstrated that compound **6** resulted in increased expression of LC3B-II(Fig. 3). These results indicate that compound **6** could trigger autophagy in HCT116 cells. Thus, the inhibitors of PDK1 could induce autophagosome formation in HCT116 cells *via* suppressing its downstream Akt/mTOR.

4. Conclusions

Three previously undescribed iridoids (**1–3**) and four known analogues (**4–7**) were isolated from the roots of *Valeriana jatamansi*. The cytotoxicity of all isolates was evaluated. Compounds **5–7** exhibited selective cytotoxicity to colon cancer cells with an IC_{50} value of 9.3, 1.7 and 2.2 μM, respectively. Based on a mechanistic study involving the Akt/mTOR signaling pathway, the cytotoxicity effect of **6** was due to Akt/mTOR activation blockade *via* inhibition of PDK1 phosphorylation. In particular, compound **6** could induce autophagosome formation in HCT116 cells *via* suppressing its downstream Akt/mTOR. Therefore, compound **6** might be a potential candidate for treatment of colon carcinoma. Meanwhile, the compounds **5** and **7** with active effect and different scaffolds are worthy of further investigation in the follow-up study.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary material

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