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The sesquiterpene lactone polymatin B from *Smallanthus sonchifolius* induces different cell death mechanisms in three cancer cell lines

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ABSTRACT

A 8β-angeloyloxy-9α-hydroxy-14-oxo-acanthospermolide and five known melampolide sesquiterpene lactones (uvedalin, enhydrin, polymatin B, sonchifolin, and fluctuanin) were isolated from the leaves of *Smallanthus sonchifolius*. The compounds were identified by 1D-, 2D-NMR, HRMS, IR and UV analyses. *In vitro* cytotoxicity assays (MTT) showed that these sesquiterpene lactones display poor cytotoxic effects on peripheral blood mononuclear cells (PBMC) of healthy human subjects, whereas a strong cytotoxicity was observed in leukemia and pancreas cancer cells. For the mechanism of action of polymatin B, oxidative stress seems to be involved. Interestingly, reactive oxygen species (ROS) formation mainly induced different effects: apoptosis in CCRF-CEM cells, necroptosis in CEM-ADR5000 cells through induction of RIP1K, neither apoptosis nor necroptosis in MIA-PaCa-2 cells. Additionally, cells also died partly by necrosis.

1. Introduction

Smallanthus sonchifolius (Poepp. & Endl.) H. Rob. (Asteraceae), commonly known as "yacon", is an herbaceous perennial plant native of the Andes and mostly known for its large edible tuberous roots (Lachman et al., 2003). Previous biological studies have demonstrated anti-inflammatory, antifungal, and antibacterial activities (Inoue et al., 1995; Lin et al., 2003). A wide variety of compounds including different phenolic acids (protocatechuic, chlorogenic, caffeic and ferulic acids), essential oil, ent-kaurenoic acid and related diterpenoid substances are known from leaves of "yacon" (Mercado et al., 2010; Ojansivu et al., 2011). In addition, phytochemical studies have shown the presence of bioactive sesquiterpene lactones (SLs) of the melampolide-type (Frank et al., 2013; Genta et al., 2010).

Sesquiterpene lactones are a large group of secondary plant metabolites mostly known from the Asteraceae family (Zhang et al., 2005). They have been described as the active constituents from several medicinal plants traditionally used to treat inflammatory diseases. However, they possess not just anti-inflammatory activity, but a rather broad spectrum of biological activities, including antibacterial, anthelmintic, uterus contracting, and antimalarial activities (Merfort, 2011). In recent years, the anticancer properties of these compounds have attracted a great interest and extensive research has been carried out to characterize the molecular mechanisms, and their potential use as chemopreventive and chemotherapeutic agents (Kreuger et al., 2012).

Some SL-derived drugs, for example those from thapsigargin, artemisinin, and parthenolide, have reached cancer clinical trials (Ghantous et al., 2010). In the case of parthenolide, the derivatization to a more hydrophilic form, diaminomethylparthenolide (DMAPT or LC-1), has enhanced its therapeutical potential. Furthermore, the ability of DMAPT to selectively eradicate acute myeloid leukemia stem cells led to the initiation of an ongoing phase I clinical trial for its use in hematologic malignancies in the UK (Ghantous et al., 2013).

The aim of this work was to evaluate the *in vitro* cytotoxicity of the SLs enhydrin (**1**), uvedalin (**2**), polymatin B (**3**), sonchifolin (**4**), 8β-angeloyloxy-9α-hydroxy-14-oxo-acanthospermolide (**5**), and fluctuanin (**6**) on three cancer cell lines – the T-cell acute lymphoblastic leukemia cell line (CCRF-CEM), the doxorubicin

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resistant T-cell leukemia cell line (CEM-ADR5000), the pancreatic carcinoma cell line (MIA PaCa-2)–, and on peripheral blood mononuclear cells (PBMC) from healthy human subjects. The isolation of compounds (**1**–**3**) has previously been published (Frank et al., 2013), compounds **4** and **6** have been re-isolated from *S. sonchifolius* and **5** is reported for the first time as a natural product. Polymatin B (**3**) was selected for further investigations on its possible mode of action.

2. Results and discussion

The leaves of *S. sonchifolius* were extracted by maceration with CH_2Cl_2 to obtain the organic extract (OE). This extract was fractionated by column chromatography (CC) on silica gel and afforded compounds **4** and **5**. Compound **4** was identified on the basis of spectroscopic data (HRMS, 1D- and 2D-NMR) as sonchifolin (Inoue et al., 1995).

Compound **5** was obtained as a white powder. Its molecular formula $C_{20}H_{24}O_6$ accounted for nine degrees of unsaturation followed from its HRMS spectrum. The IR spectrum showed absorptions at 3530 cm^{-1} (OH), 1782 cm^{-1} (lactone), 2725, 1668 and 1635 cm^{-1} (unsaturated aldehyde), 1750 and 1656 cm^{-1} (unsaturated ester). The ^1H NMR spectrum exhibited signals attributed to a germacranolide-type compound bearing an angelate ester, because it was similar to the spectra of related melampolides (Cartagena et al., 2000; Macías and Fischer, 1992; Macías et al., 1993). The presence of a 1,10-*cis*-double bond with an aldehyde group at C-10 followed from the chemical shifts of H-1 (δ 6.66,dd) and H-14 (δ 9.50, d) (Herz and Kalyanaraman, 1975). The *trans*-configuration of the 4,5-double bond was deduced from the typical chemical shifts of H-5 (δ 4.94, br d) and H-6 (δ 5.09, t), as well as a large coupling constant for $J_{6,7}$ of 10 Hz (Bohlmann et al., 1979) which agrees with a dihedral angle of approximately 180° between H-6 and H-7. In heliangolides with a 6,12-*trans*-lactone moiety and a *cis*-configured 4,5-double bond, a dihedral angle of about 120° can be calculated between H-6 and H-7 which is in accordance with a coupling constant of $J_{6,7}$ (1.5–3.5 Hz) (Castro et al., 1989; de Hernández et al., 1997, 1999; de Gutiérrez et al., 2001; de Heluani et al., 1989). This small coupling constant is also reported for *cis*,*cis*-germacranolides (Stokes et al., 1992).

The two exo-methylene-γ-lactone hydrogens H-13a and H-13b appeared as doublets at 6.28 ppm and 5.70 ppm with J = 3.2 Hz and 3.0 Hz respectively, clearly indicating the occurrence of a *trans* lactonized melampolide (Diaz et al., 1992; Herz and Sharma,1975; Samek, 1970). The signal at δ 6.52 (dd) is typical for a proton attached to a carbon supporting an ester group (angeloyloxy) and was assigned to H-8. The small coupling constant between H-7 and H-8 (1.5 Hz) indicated that the ester residue at C-8 is β-oriented. The chemical shift of H-9 at 3.99 ppm (dd) is typical for an α-oriented hydroxyl group at C-9 (de Pedro et al., 2003; Le Van and Fischer, 1979). Moreover, the α-configuration at C-9 is confirmed by the coupling constant $J_{8,9}$ (8.5 Hz) and a W-type coupling between H-9β and H-14 (J = 2.0 Hz) (Cartagena et al., 2000). The ^{13}C NMR spectrum showed 20 carbons whose assignments were achieved with the aid of DEPT, HSQC, and HMBC experiments, consisting of one conjugated aldehyde (δ 195.2), two carbonyl carbons (δ 169.2, 167.7), three methylene carbons (δ 121.6, 36.8, 26.4), three olefinic methine carbons (δ 155.2, 139.9, 126.9), four methine carbons including three oxygenated signals (δ 75.3, 71.2, 70.7, 51.3), four quaternary olefinic carbons (δ 144.6, 137.6, 134.5, 126.8) and three methyl carbons (δ 20.5, 16.9, 15.9). Comparison of the ^1H and ^{13}C NMR spectra of compound **5** with those of polymatin A (de Pedro et al., 2003; Le Van and Fischer 1979; Serra-Barcellona et al., 2014) clearly shows that compound **5** is the aldehyde analog at C-14. A C-9 epimer of **5** has been isolated from *Grazielia intermedia* (Bohlmann et al., 1981; López Pérez et al., 2007) as evidenced by the large difference in chemical shift for H-9 (3.99 ppm vs. 5.06 ppm), the smaller value for $J_{8,9}$ (8.5 Hz vs. 5.0 Hz) and the missing W-coupling with H-14. A C-9 O-ethyl derivative of **5** has been isolated from *S. sonchifolius* cultivated in Korea (Hong et al., 2008). Based on the above spectroscopic data, the structure of **5** was therefore deduced to be 8β-angeloyloxy-9α-hydroxy-14-oxo-1(10)E,4E,11(13)-germacratrien-

Table 1
Cytotoxic activity expressed as IC_{50} values (μM) of sesquiterpene lactones **1**–**6** isolated from *Smallanthus sonchifolius* against three tumor cell lines and PBMCs after 24 h of incubation using the MTT assay.

Compound	CCRF-CEM[a]	CEM-ADR5000[a]	MIA-PaCa-2[a]	PBMC[a]	tPSA (Å^2)	*C*log*P*
1	3.6 ± 0.3	26.6 ± 1.3	12.6 ± 0.2	>20	117.7	3.8
2	9.2 ± 2.2	20.1 ± 1.4	21.2 ± 2.0	>20	130.3	2. 6
3	0.8 ± 0.02	1.3 ± 0.2	3.7 ± 0.2	>20	105.2	3.8
4	3.1 ± 0.1	3.1 ± 0.2	7.4 ± 0.6	>20	78.9	4.1
5	2.2 ± 0.2	6.7 ± 0.3	8.9 ± 0.4	>20	89.9	2.4
6	0.6 ± 0.1	1.4 ± 0.09	4.4 ± 0.5	>20	117.7	2.4
CPT[b]	0.08 ± 0.02	0.3 ± 0.1	>10	>10	n.d.	n.d.
Doxo[b]	0.5 ± 0.03	>10	4.8 ± 0.6	>10	n.d.	n.d.

[a] The data represent the IC_{50} values (μM) ± s.d. obtained from the non-linear regression of three independent experiments.
[b] Camptothecin (CPT) and doxorubicin (Doxo) were used as positive controls. tPSA: topological polar surface area. *C*log*P*: partition coefficient. n.d.: not determined.

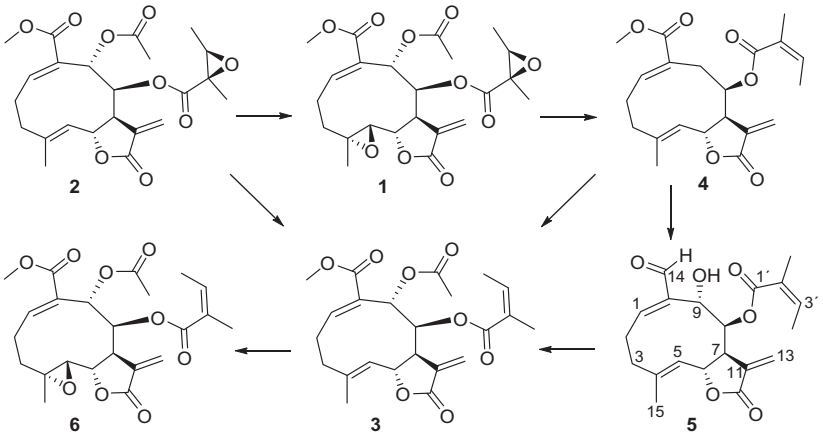

Fig. 1. Structures of the SLs from *S. sonchifolius*. Each arrow represents a structural modification that enhances the cytotoxic activity in CCRF-CEM and MIA-PaCa-2 cells.