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Cytotoxicity of abietane diterpenoids from *Salvia multicaulis* towards multidrug-resistant cancer cells

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ABSTRACT

Diterpenoids salvimulticanol (**1**) and salvimulticaoic acid (**2**) together with known diterpenoid (**3–6**) were isolated from *Salvia multicaulis*. Structures were elucidated by spectroscopic techniques including HRESIMS as well as 1D-, and 2D-NMR. In-vitro cytotoxicity was assayed against human cancer cell lines. As several metabolites exhibited activity against drug-resistance lines, compounds were screened against a panel of human drug-sensitive and multidrug-resistant cancer lines. A proposed biosynthetic pathway for these new diterpenoids (**1–2**) as well as the cytotoxic structure-activity relationship of all identified compounds were discussed. Compound **1** and **6** showed the most potent cytotoxicity with IC_{50} 11.58 and 4.13 towards leukemia cell lines CCRF-CEM and CEM-ADR5000, respectively.

1. Introduction

Salvia is one of the largest genera in the family Lamiaceae (Labiatae), consisting of > 900 species that are widely dispersed throughout the world, including the Mediterranean region, South-East Asia and Central and South America [1–3]. Most of the *Salvia* species are pharmacologically active and have been widely used in folk medicine for > 60 different ailments ranging from aches to epilepsy. Target treatments include colds, bronchitis, tuberculosis, obesity, diabetes, depression, dementia and menstrual disorders [4–7]. *Salvia* species are renowned for their abundance of flavonoids, phenolics, terpenoids and steroids, most of which have a broad spectrum of biological activity including antimicrobial, antioxidant, anti-inflammatory, anticancer and antiviral activities [7–9].

The diterpenoids from *S. multicaulis* roots showed significant activity against *Mycobacterium tuberculosis* strain H37Rv [10]. Additionally, Essential oil of *S. multicaulis* showed antimicrobial activity against several strain [11–13].

Multidrug resistance (MDR) presents a major disruption effect for cancer chemotherapy. Cancer cell MDR involves several members of the adenosine triphosphate binding cassette (ABC) transporters such as ABCB1, ABCC1 and ABCG2 that can effectively efflux anti-cancer drugs [14, 15]. The acquisition of MDR is usually mediated by overexpression of ABC transporters that precipitates in the failure of cancer chemotherapy [16–19]. Thus, identification of cytotoxic drugs unaffected by ABC drug resistance is being sought. The structural diversity of natural compounds from medicinal plants provides a rich source of potent metabolites to block the MDR phenotype [20–24]. As part of our research to investigate and biologically evaluate the wild Egyptian plants [25–32], herein, an organic extract of *S. multicaulis* was chemically analyzed for diterpenoid (**1–6**) (Fig. 1) and purified compounds were assayed for cytotoxic activity against sensitive and resistance multidrug-resistant cancer cells. Structure-activity relationships and a biosynthetic pathway for diterpenoid assembly are proposed.

2. Experimental

2.1. General procedure

Specific rotation was measured with a JASCO P-2200 polarimeter

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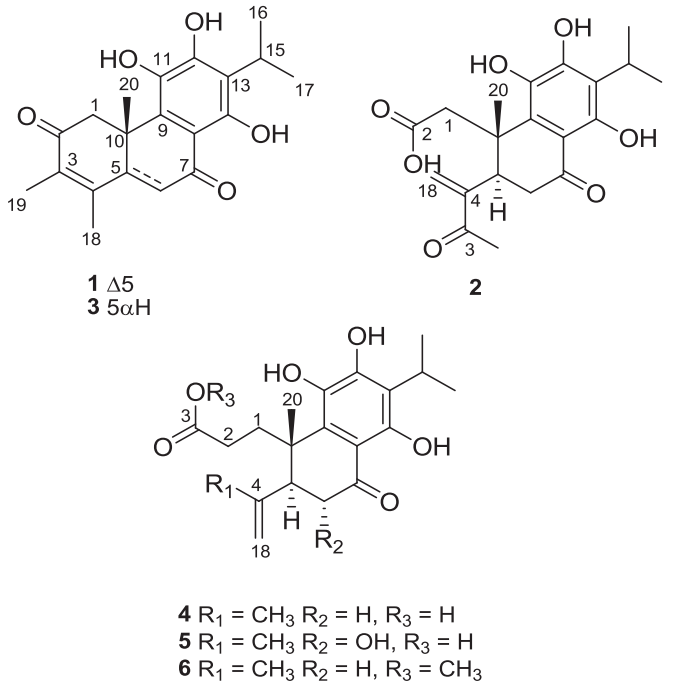

Fig. 1. Structures of the isolated compounds (**1–6**) from *S. multicaulis*.

(JASCO Corporation, Tokyo, Japan) and IR spectra were collected on a JASCO FT/IR-6300 spectrometer (JASCO Corporation, Tokyo, Japan). HRESIMS was obtained with a Q-ToF ULTIMA-III quadrupole TOF mass spectrometer (Waters, Eschborn, Germany). The ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded in $CDCl_3$ on a JEOL ECA- 600 spectrometer (JEOL Ltd., Tokyo, Japan) with tetramethylsilane (TMS) as internal standard. Purification was run on a Shimadzu HPLC system equipped with a RID-10A refractive index detector and compound separation was performed on YMC-Pack ODS-A (YMC CO. LTD., Tokyo, Japan, 250 × 4.7 mm i.d., 5 μm) and 250 × 10 mm i.d., 5 μm) columns for analytical and preparative separation, respectively. Chromatographic separation included normal phase silica gel 60 (230–400 mesh, Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia Co. Tokyo, Japan) were used for column chromatography. TLC analysis was performed on pre-coated silica gel plates (Kieselgel 60 F_{254}, 0.25 mm, Merck, Darmstadt, Germany) and spots were detected by spraying with 10% H_2SO_4 solution followed by heating.

2.2. Plant material

The air-dried aerial parts of *S. multicaulis* Vahl. were collected from South Sinai, Egypt in May 2015. A voucher specimen was deposited in the Herbarium of Saint Katherine protectorate, Egypt, as well as the herbarium of the National Research Centre (voucher No. 310), Cairo, Egypt. The collection took place under the permission of Saint Katherine Protectorate for scientific research.

2.3. Extraction and isolation

Aerial parts (1.0 kg) were powdered and extracted with CH_2Cl_2:MeOH (1:1) at room temperature. The extract was concentrated in vacuo to obtain a gummy residue (110 g). The concentrated crude extract was fractionated on silica gel flash CC (5 × 60 cm) and eluted with gradient solvents of increasing polarity starting with (100%) *n*-hexane followed by a gradient of *n*-hexane/ethyl acetate up to 100% ethyl acetate. Eighteen fractions were collected and pooled together according to the TLC profile. Vanillin-sulphuric acid spray reagent was used for compound spots detection. Similar fractions were pooled according to their chromatographic properties to yield seven collected fractions as the following: A (15 g), B (5.5 g), C (10.5 g), D (14 g), E (12 g), F (5.5 g), G (6.5 g). **Fraction D** (14 g) was subjected to further fractionation on ODS column (3 × 60 cm) using 80:20% (MeOH: H_2O) and finally wash with 100% MeOH. The obtained sub-fraction was subjected to isolation and purification by a reversed phase HPLC (20 × 250 cm) using MeOH:H_2O (9:1, 2.5 L) with flowrate 3 mL/min to afford compound (**6**, 20 mg). **Fraction E** (12 g) was also subjected to further fractionation on ODS column (3 × 60 cm) using 75:25% (MeOH: H_2O) and finally washed with 100% MeOH. The obtained fraction was further purified by a reversed phase HPLC using MeOH: H_2O (8:2, 2.5 L) with flowrate 6 mL/min to afford compounds (**1**, 10.5 mg) and (**3**, 14.0 mg). **Fraction F** (5.5 g) was purified by a reversed phase HPLC using MeOH: H_2O (7:3, 2.5 L) with flowrate 6 mL/min to afford compounds (**2**, 8 mg) and (**4**, 12 mg). **Fraction G** (6.5 mg) was purified by a reversed phase HPLC using MeOH:H_2O (50:50%, 2.5 L) with flowrate 6 mL/min to afford compound (**5**, 8.5 mg).

2.3.1. Compound 1

11,12,14-trihydroxy-19(4 → 3)-abeo-3,5,8,11,13-abietapentaen-2,7-dione (salvimulticanol). Colorless oil; $[\alpha]_D^{25}$ + 318.0 (c 0.01, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 341.1383 $[M-H]^-$; (calcd. for $C_{20}H_{22}O_5$, 341.1394).

2.3.2. Compound 2

11,12,14-trihydroxy-3,7-dione-2,3-seco-4(18),8,11,13-abietatetraen-2-oic acid (salvimulticaoic acid). Colorless oil; $[\alpha]_D^{25}$ 38.8 (c 0.01, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 359.1493 $[M-OH]^-$; (calcd. for $C_{20}H_{24}O_7$, 359.1489).

2.3.3. Cell culture and treatment conditions

The drug-sensitive leukemia cell line CCRF-CEM and its multidrug-resistant P-glycoprotein-overexpressing subline CEM/ADR5000 (treated once per week with 5000 ng/mL doxorubicin) were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (v/v).

Breast cancer cells MDA-MB-231-pcDNA3 and their multidrug-resistant subline MDA-MB-231-BCRP clone 23 (treated once per week with 300 ng/mL geneticin), colon cancer cells HCT116 ($p53^{+/+}$) and

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for **1** and **2.**

No.	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1a	2.40 d (17.0)	45.5	2.22 d (17.0)	41.9
b	4.18 d (17.0)		2.71 d (0.7)	
2	–	198.6	–	166.1
3	–	136.2	–	198.7
4	–	147.5	–	146.2
5	–	160.2	2.43 m	39.2
6	6.53 s	124.4	2.91 dd (14.7,18)	39.3
			3.82 br d (3.6, 14.7)	
7	–	189.1	–	200.9
8	–	108.0	–	106.4
9	–	131.5	–	128.9
10	–	42.1	–	36.2
11	–	134.7	–	130.2
12	–	149.3	–	150.0
13	–	119.8	–	121.5
14	–	157.7	–	160.6
15	3.44 m	24.9	3.54 m	24.2
16	1.38 d (7.0)	20.5	1.31 d (7.0)	19.9
17	1.39 d (7.0)	20.5	1.32 d (7.0)	20.0
18	2.21 s	17.6	5.96 br s	129.7
			6.52 br s	
19	2.00 s	12.0	2.42 s	25.8
20	1.62 brs	25.1	1.17 s	18.1