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.

Keywords
Salvia multicaulis
Lamiaceae
Abietane diterpenoids
Cytotoxic activity
Multidrug resistance

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], [2], [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], [5], [6], [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], [8], [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], [12], [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], [17], [18], [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], [21], [22], [23], [24]]. As part of our research to investigate and biologically evaluate the wild Egyptian plants [[25], [26], [27], [28], [29], [30], [31], [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 (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 F254, 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; $[\alpha]^{25}_D$ + 318.0 (c 0.01, MeOH); ^1H 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; $[\alpha]^{25}_D$ 38.8 (c 0.01, MeOH); ^1H and ^{13}C NMR data,

see Table 1; HRESIMS *m/z* 359.1493 $[M\text{-}OH]^-$; (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 their knockout clone HCT116 ($p53^{-/-}$) (treated once per week with 800 ng/mL geneticin), glioblastoma cells U87MG and their resistant subline U87MG. ΔEGFR (treated once per week with 800 ng/mL geneticin) and HEK-293and HEK-293-ABCB5 were cultured in DMEM medium supplemented with 10% FBS, 1% penicillin-streptomycin (v/v). The cells were kept in a humidified atmosphere with 5% CO_2 at 37 °C.

2.3.4. Resazurin cytotoxicity assay

The cytotoxicity of isolated compounds was determined by resazurin reduction assay (O'Brien et al., 2000). The principle of the assay is based on the reduction of resazurin by actively metabolic living cells to highly fluorescent dye resorufin. Suspension cells (1×10^4 cells/well) were seeded onto 96-wells plate in a volume of 100 μL and varying concentrations for the generation of concentration-response curves of isolated metabolites, were added immediately to reach total volume of 200 μL. Adherent cells were incubated 5×10^3 cells/well in 96-wells plate in a volume of 100 μL for overnight to let them attach. Afterwards varying concentrations of crude extract were added in the same manner as mentioned above. After 72 h, 20 μL of 0.01% w/v resazurin (Sigma-Aldrich, Taufkirchen, Germany) was added to each well, cells were incubated for 4 h at 37 °C. Fluorescence at excitation wave length 544 nm and emission at 590 nm was measured using Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany). Each assay was repeated three independently with six replicate each. Fifty percent inhibitory concentration (IC_{50}) values were calculated using the concentration-response curve fit to the nonlinear regression model using GraphPad Prism v6.0 software (GraphPad Software Inc., San Diego, CA, USA). All IC_{50} values are expressed as mean ± standard deviation (SD).

3. Results and discussion

Chromatographic fractionation and purification of a dichloromethane/methanol (1:1) extract of the aerial parts of *S. multicaulis* using normal and reversed phase chromatography afforded new diterpenoid compounds (1-2), in addition to known compounds (3-6) (Fig. 1).

Compound 1 was obtained as a colorless oil with an optical rotation of $[\alpha]^{25}_{D}$ + 318.0 (c 0.01, MeOH). The molecular formula, $C_{20}H_{22}O_5$ was determined on the basis of HR-ESI-MS analysis (*m/z* 341.1383 $[M-H]^-$(calcd. for $C_{20}H_{22}O_5$, 341.1394), indicating 10 degrees of unsaturation as a double bond equivalent of which three were accounted for by the presence of a tricyclic skeleton while the remaining seven degrees of unsaturation suggested the presence of seven double bonds. ^{13}C NMR and DEPT spectral analyses revealed the presence of five methyls, one methylene, two methines (one olefinic) and 12 quaternary carbons (two keto and 10 olefinic), indicating 20 carbon molecules with the presence of a six-membered aromatic system (see Table 1). This proposed skeleton combined with two olefinic methyl singlet signals at δ_H 2.21 (3H, s, Me-18) and 2.00 (3H, s, Me-19) as well as a methyl signal at δ_H 1.62 (3H, brs, Me-20) suggested an abietane-type diterpenoid [29, 30]. Characteristic proton NMR signals for an isopropyl group were observed at δ_H 1.38 (3H, d, J = 7.0 Hz, H-16), δ_H 1.39 (3H, d, J = 7.0 Hz, H-17), and δ_H 3.44 (1H, m, H-15). HMBC correlations observed between H-16/C-15, H-17/C-15, H-16/C-13 and H-17/C-13 were consistent with the propyl attachment at C-13 (Fig. 2); a downfield singlet signal at δ_H 6.53 (1H, s) was assigned to H-6 and two doublets at δ_H 2.40 (1H, d, J = 17.0 Hz, H-1a) and 4.18 (1H, d, J = 17.0 Hz, H-1b) to H_2-1. The locations of the carbonyl groups at C-2 and C-7 were supported by observed correlation between H-1/C-2, H-19/C-2 and between H-6/C-7 in HMBC analysis (Fig. 2). The β configuration of methyl group at C-10 (H_3-20, δ_H 1.62, brs) was assigned based on biogenetic precedent and was consistent with previously reported NMR chemical shift data for similar abietane type diterpenoids [[33], [34], [35], [36], [37], [38]]. From the above data, 1 was identified as 11,12,14-trihydroxy-19(4 → 3)-abeo-3,5,8,11,13-abietapentaen-2,7-dione (salvimulticanol, 1), a new natural compound.

Compound 2 was isolated as a yellowish oil with an optical rotation of $[\alpha]_D^{25}$ + 38.8 (c 0.01, MeOH) The HRESIMS spectrum (*m/z* 359.1493 $[M-OH]^-$ (calcd. For $C_{20}H_{24}O_7$, 359.1489) corresponded to a molecular formula of $C_{20}H_{24}O_7$ with 9 degrees of unsaturation as double bonds equivalents of which, five were accounted for the presence of a bicyclic skeleton containing a six-carbon aromatic system. The ^{13}C NMR and DEPT spectra showed 20 carbon atoms and classified as, four methyls, three methylenes (one olifenic), two methines and eleven quaternary carbons. Both ^{1}H and ^{13}C NMR spectra of

1 and 2 were similar (Table 1), with three diagnostic modifications. [i] Two protons shifted downfield to broad singlets at δ_H5.96 (1H, brs, H-18a) and 6.52 (1H, brs, H-18b) being attached to C-18 at δ_C 129.7 in 2 instead of H-18 at δ_H 2.21 (1H, s) and C-18 at δ_C 17.6 in 1. This suggested a replacement of Me-18 in 1 by exomethylene group in 2. [ii] An absence of a C-5/C-6 double bond in 2, which was confirmed by the appearance of H-5 at δ_H 2.43 (1H, m) and C-5 at δ_C 39.2 in addition to the presence of two new signals set at δ_H 2.91 (IH, dd, J = 14.7, 18.0 Hz, H-6a), 3.82 (IH, dd, J = 3.6, 14.7 Hz, H-6b) and C-6 at 39.3 δ_C with respect to those of 1 at δ_C 160.2 (C-5), δ_H 6.53 (1H,s, H-6) and δ_C 124.4 (C-6). And [iii] a down-field shift of the C-3 resonance from δ_C 136.2 in 1 to δ_C 198.7 in 2 accompanied by a C-2 shift from δ_C 198.6 in 1 to upfield position at δC166.1 in 2, and the appearance of Me-19 at δ_H 2.42 (3H, s) in 2 instead of δ_H 2.00 in 1, indicating the presence of acetyl group at C-4 together with carboxyl group at C-1. From these observations, the structure of 2 has 2,3-seco-abietane rearranged diterpenoid skeleton. The location of the carboxyl group at C-1 and acetyl group at C-4 was confirmed from HMBC correlations observed between H-1/C-2 and H-19/C-3, respectively (Fig. 2). Two and three bond correlations between H-5/C-3, H-5/C-4, H-5/C-6, H-6/C-4, H-6/C-5, H-6/C-7, H_2-18/C-3, H_2-18/C-4 and H_2-18/C-5 were further supported the assignment of H-5, H-6 and H-18 (Fig. 2).

All of the abietane derivatives isolated from the same genus related to compound 2 indicated that the relative configuration of H_3-20 and H-5 to be in β and α orientating, respectively [35, 36, 39]. All of the above data were compatible with 2,3-seco-abietane skeleton of 2 and was elucidated as, 11,12,14-trihydroxy-3,7-dione-2,3-seco-4(18),8,11,13-abietatetraen-2-oic acid (salvimulticaoic acid, 2).

Four previously isolated compounds were identified by comparison of published spectral data including: 2-oxocandesalvone, 3 [35], candesalvone B, 4 [36], 6-β-hydroxycandesalvone B, 5 [36] and candesalvone B methyl ester, 6 [39].

Geranylgeranyl diphosphate (GGPP) is considered the main precursor for biosynthesis of diterpenoid compounds, and recently miltradiene was shown to be the precursor of a vast array of phenolic abietane diterpenoids through the incorporation of oxygen catalyzed by cytochromes P_{450} enzyme (PYP), (Fig. 3) [40]. Recent studies suggested that many complex reactions in the plant, including aromatization are enzymatically catalyzed and the aromatic intermediate abietatriene simply hydroxylated by a specific cytochrome P_{450} enzyme, (Fig. 3) [41, 42]. Thus, the biosynthesis of 1 is proposed to go through an aromatization of the C ring, hydroxylation and oxidation reactions, as well as rearrangement reactions in the A and B rings (Fig. 3). Compound 2 is proposed to be generated biosynthetically from 1 via a condensation of the carbonyl

group at C-1 with acetyl-CoA, followed by a rearrangement reaction in ring A, oxidation of acetyl group and reduction of $\Delta^{5,6}$ bond (Fig. 3).

Cancer is one of the most prevalent causes of deaths globally [43]. Numerous plant extracts have been shown to possess potential anticancer activity. In the present study, the isolated compounds (1-6) were initially screened in vitro against two cancer cell lines: human acute lymphocytic leukemia (CCRF-CEM) and childhood T acute lymphoblastic leukemia (CEM-ADR5000) at five concentrations (0.01-100 μM) using the Resazurin reduction assay [44]. Cytotoxic activity was observed for the doxorubicin-resistant CEM-ADR5000 cells with an IC_{50} values ranging from 4.1-21.60 μM. This compound appears to be more potent than for CCRF-CEM cells which exhibited an IC_{50} ranging from 11.6-31.5 μM, (see Table 2). The highest cytotoxicity was observed with 6 against CEM-ADR5000 cells showing an IC_{50} 4.1 μM, followed by 1, 5, 3, 4 and 2 with respective IC_{50} values of 8.36, 10.77, 11.37, 19.61 and 21.60 μM. In contrast, 3 was the most active against CCRF-CEM cells with an IC_{50} of 11.58 μM, followed by 1, 6, 5, 2, and 4 with IC_{50} values; 15.32, 20.95, 21.54, 28.28 and 31.52 μM, respectively. Compounds 1, 3, 5, and 6 were efficacious against both CEM-ADR5000 and CCRF-CEM cells. Compound 3 showed IC_{50} values ranging from 1.30-23.84 μM for cell lines MDA-MB-231-pcDNA, MDA-MB-231-BCRP clone 23, U87MG, U87MG.ΔEGFR, HCT116 ($p53^{+/+}$), HCT116 ($p53^{-/-}$), HEK-293, and HEK-293-ABCB5 cells (see Table 2). Other biologically active metabolites included 1 with and an IC_{50} range of 1.62-38.95 μM, 6with an IC_{50} range of 1.72- > 100 μM and 5 with an IC_{50} range of 1.84 - >100 μM. Both U87MG and U87MG.ΔEGFR cells were resistant to 5 and 6with IC50 (>100 μM), (Fig. 4, Fig. 5, Table 2, Table 3).

All isolated compounds (1-6) belongs to the aromatic abietane diterpenoids possessing an aromatic C-ring with *p*- and *o*-phenolic hydroxyl groups (catechol) together with the characteristic isopropyl moiety that are the essential structural requirements or pharmacophore required for these compounds to show cytotoxic activity against human cancer cell lines [45]. Also, all compounds in our current study contained an endocyclic α,β-unsaturated carbonyl functional group at C-7 on B-ring, which plays an important role as a key pharmacophore for exhibiting cytotoxic activity [8, 46, 47]. Previous studies also demonstrated that the α,β-unsaturated carbonyl functional group facilitates the alkylation of cellular thiol groups in a Micheal type addition reaction causing cell damage and enhancing the cytotoxic activity [[48], [49], [50]]. In the current study, 3 has the same chemical structure as 1, except for a saturated $\Delta^{5,6}$ double bond in 3 (Fig. 1). Cells of CCRF-CEM, MDA-MB-231-pcDNA, MDA-MB-231-BCRP clone 23, U87MG, U87MG.ΔEGFR, HCT116 ($p53^{+/+}$), HCT116 ($p53^{-/-}$), HEK-293, and HEK-293-ABCB5

were all more sensitive to 3 comparing with 1, demonstrating that the absence of $\Delta^{5,6}$ double bond in 3 is favorable although not a prerequisite for cell cytotoxic activity (Table 2, Table 3). Interestingly, 1 was only more cytotoxic than 3 against the CEM-ADR5000 cells (Table 2), that can be attributed to the conjugation of two endocyclic carbonyl groups at C-2 and C-6 with $\Delta^{3,4}$ and $\Delta^{5,6}$ double bonds. The minor difference in structures of 4, 5 and 6, can be attributed the variations in their cytotoxic activity (Fig. 1), (Table 2, Table 3). As regards the effect of C-6 substitution on the cytotoxic activity, a hydroxyl group at C-6 seems to enhance the activity against both CCRF-CEM and CEM-ADR5000 cells more than those analogs without substitution (5 vs 4). Additionally, 5 is slightly more active than 6 against both the MDA-MB-231-pcDNA and HEK-293 cells, suggesting that the analogs having carboxylic group is more cytotoxic than the corresponding methyl ester containing analogs (5 vs 6), (Table 3). In contrast, 6, more cytotoxic than 5 against the CCRF-CEM, CEM-ADR5000, MDA-MB-231-BCRP clone 23, HCT116 ($p53^{+/+}$), HCT116 ($p53^{-/-}$) and HEK-293-ABCB5 cells (see Table 2), indicating that these cells are more sensitive to the methyl ester containing analogue than the carboxylic one, while as the U87MG and U87MG.ΔEGFR cells are not sensitive to both analogs (6 vs 5).

While 1-6 easily oxidize to the corresponding quinone on the C-ring, the quinone-bearing C-ring is an essential pharmacophore requirement for a series of phenolic abietane diterpenoids to exhibit cytotoxic activity [45].

Conflict of interest

The authors declare that there is no conflict of interest.

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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) 3.82 br d (3.6, 14.7)	39.3
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 6.52 br s	129.7
19	2.00 s	12.0	2.42 s	25.8
20	1.62 brs	25.1	1.17 s	18.1

Table 2. Cytotoxicity of isolated compounds (**1**–**6**) towards CCRF-CEM and CEM-ADR5000 Human Leukemia cancer cell lines determined by the resazurin assay after 72 h incubation.

Cell lines	**Compounds**						**Doxorubicin**
	1	**2**	**3**	**4**	**5**	**6**	
	(IC_{50}, µM)						
CCRF-CEM	15.32 ± 0.29	28.28 ± 0.15	11.58 ± 0.12	31.52 ± 0.14	21.54 ± 0.16	20.95 ± 0.15	0.01 ± 0.06
CEM-ADR5000	8.36[a] ± 0.16 (0.54)	21.6[a] ± 0.14 (0.76)	11.37[a] ± 0.11 (0.001)	19.61[a] ± 0.11 (0.62)	10.77[a] ± 0.12 (0.5)	4.13[a] ± 0.10 (0.19)	66.83[a] ± 0.05 (3341)

Table 3. Cytotoxicity of compounds (1, 3, 5 and 6) showed potent collateral activities against drug-resistance CEM-ADR5000 cells towards a panel of human drug-sensitive and multidrug-resistant cancer cell lines determined by the resazurin assay after 72 h incubation. Resistance of MDA-MB-231-BCRP cells, HCT116 ($p53^{-/-}$) cells, U87MG.ΔEGFR cells, and HEK-293-ABCB5 cells towards doxorubicin as control drug has been published by us [40, 46].

Cell lines	**Compounds**			
	1	**3**	**5**	**6**
	(IC_{50}, µM)			
MDA-MB-231-pcDNA	32.01 ± 0.05	20.55 ± 0.08	83.69 ± 0.07	89.15 ± 0.08
MDA-MB-231-BCRP	26.40* ± 0.09 (0.82)	14.53* ± 0.13(0.70)	74.66* ± 0.16 (0.89)	60.44* ± 0.12 (0.67)
HCT116 ($p53^{+/+}$)	30.91 ± 0.11	19.82 ± 0.08	46.46 ± 0.10	41.02 ± 0.12
HCT116 ($p53^{-/-}$)	38.95* ± 0.13 (1.26)	19.94* ± 0.13 (1.00)	77.15* ± 0.17 (1.66)	70.43* ± 0.10 (1.71)
U87MG	30.33 ± 0.09	19.95 ± 0.12	>100	>100
U87MG.ΔEGFR	35.31* ± 0.10 (1.16)	23.84* ± 0.11 (1.19)	>100	>100
HEK-293	45.61 ± 0.09	23.08 ± 0.09	66.88 ± 0.08	74.80 ± 0.08
HEK-293-ABCB5	42.02* ± 0.09 (0.92)	19.97* ± 0.10 (0.86)	70.19* ± 0.07 (1.04)	52.51* ± 0.1 (0.70)

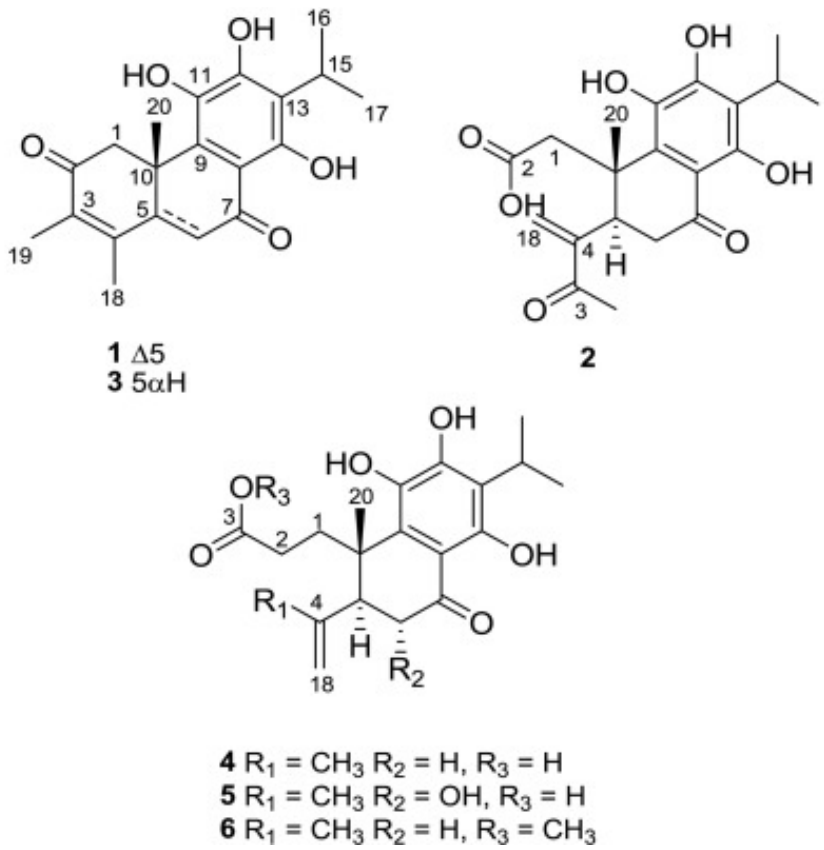

Fig. 1. Structures of the isolated compounds (**1**–**6**) from *S. multicaulis.*

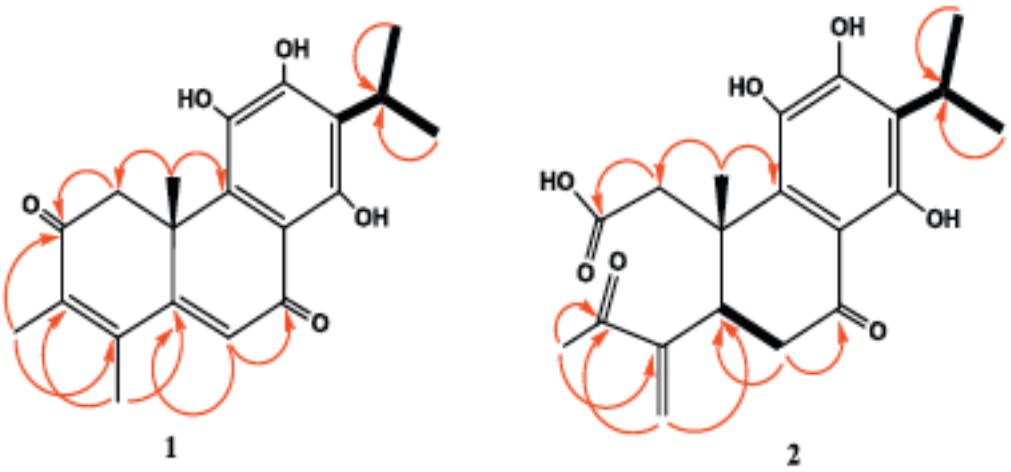

Fig. 2. Select HMBC (→) and ^{1}H-^{1}H COSY (−) correlation for **1** and **2.**

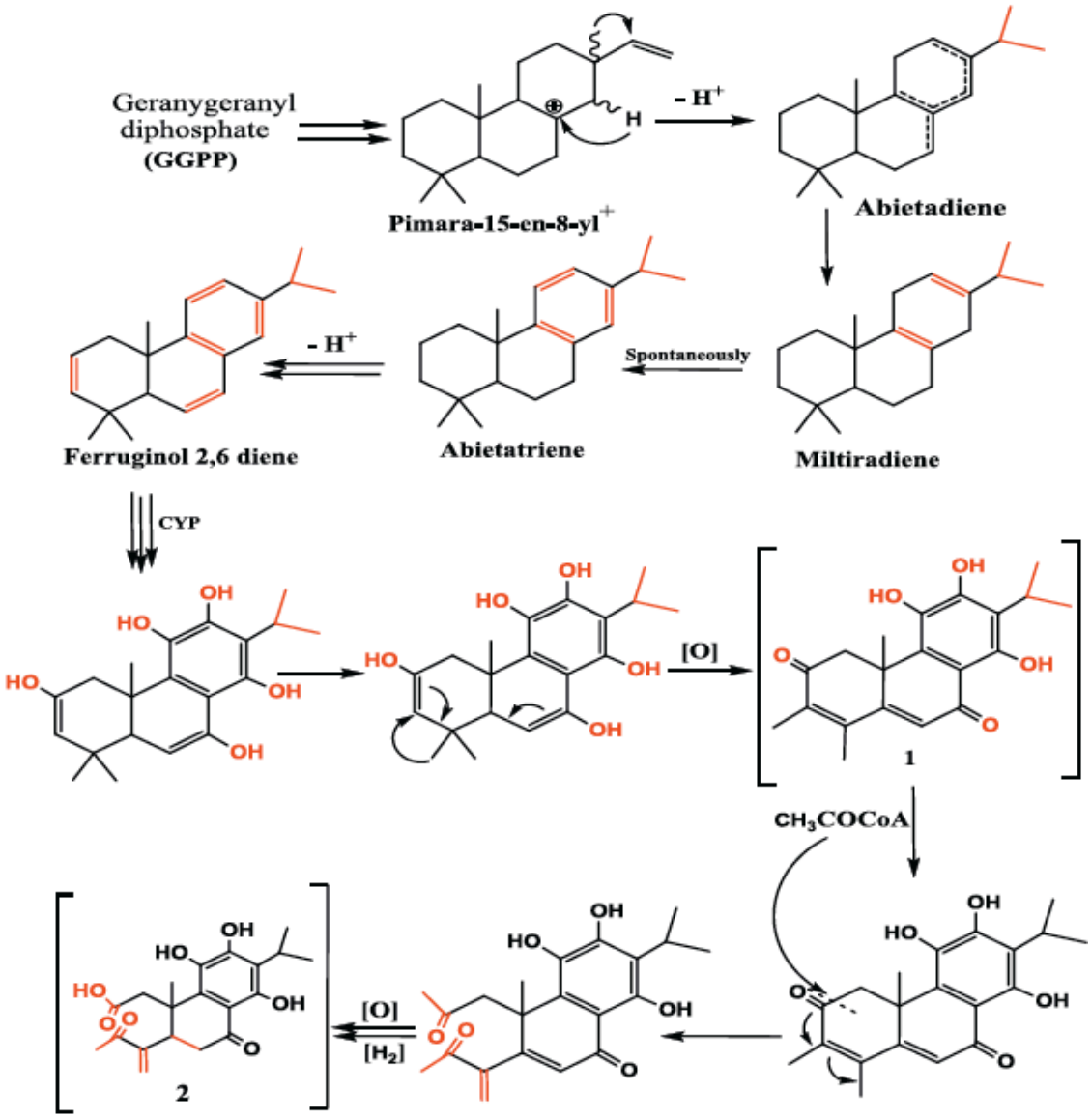

Fig. 3. Proposed scheme of biosynthesis pathway for **1**–**2.**

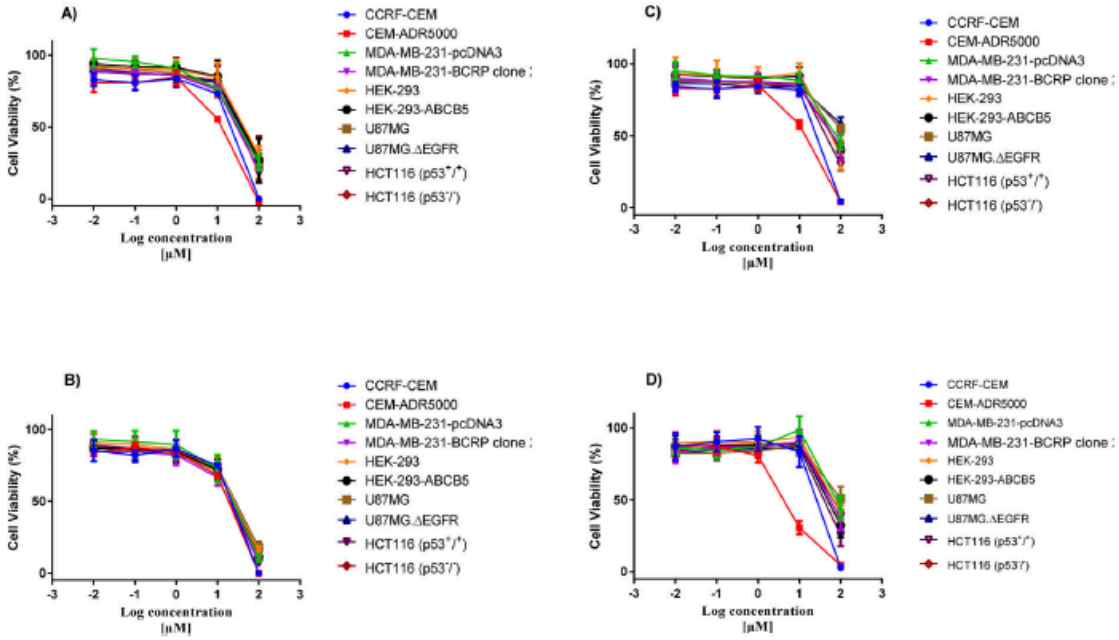

Fig. 4. Dose response curves of **1** (A), **3** (B), **5** (C) and **6** (D) towards drug-sensitive parental CCRF-CEM and their multidrug-resistant subline, CEM/ADR5000, Breast cancer cells MDA-MB-231-pcDNA3 and their multidrug-resistant subline MDA-MB-231-BCRP clone 23, colon cancer cells HCT116 ($p53^{+/+}$) and their knockout clone HCT116 ($p53^{-/-}$), glioblastoma cells U87MG and their resistant subline U87MG.ΔEGFR and HEK-293 and HEK-293-ABCB5. as determined by the resazurin assay. Mean values and standard deviations of each three independent experiments with each six parallel measurements are shown.

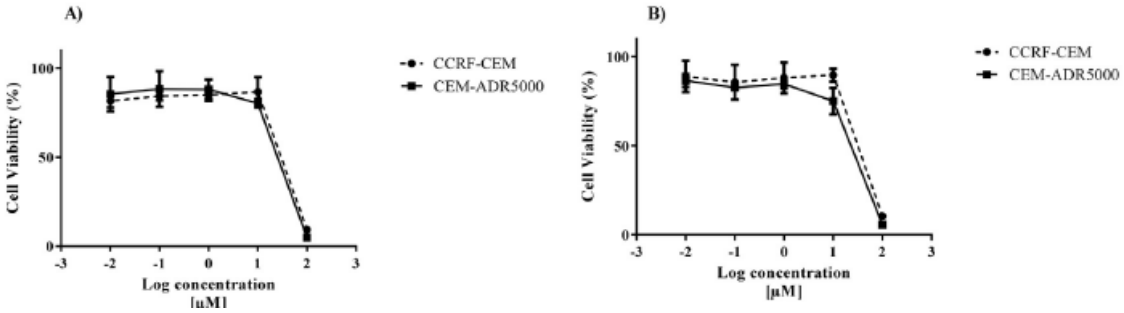

Fig. 5. Dose response curves of **2** (A) and **4** (B) towards drug-sensitive parental CCRF-CEM tumor cells and their P-glycoprotein-expressing, multidrug-resistant subline, CEM/ADR5000 as determined by the resazurin assay. Mean values and standard deviations of each three independent experiments with each six parallel measurements are shown.