Three New Ingol Diterpenes from *Euphorbia nivulia*: Evaluation of Cytotoxic Activity[1)]

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The latex of *Euphorbia nivulia* afforded three new ingol diterpenes, 3-acetyl-8-methoxyl-7-angolyl-12-hydroxylingol (7), 3,12-diacetyl-7-hydroxy-8-methoxylingol (8), and 3,12-diacetyl-7-angolyl-8-hydroxylingol (9) along with five known ingol diterpenes 2—6 and a known triterpene cyclonivulinol (1). Their structures were established by means of spectroscopic analysis. Diterpenes 2—9 were evaluated for their cytotoxic activity.

Key words *Euphorbia nivulia*; Euphorbiaceae; ingol diterpenoids; cytotoxic activity

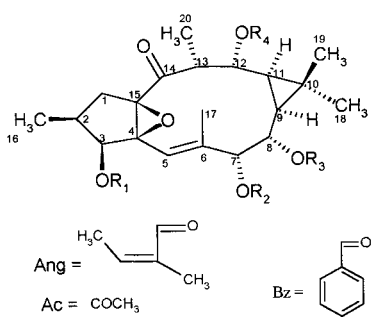
The genus *Euphorbia* is the largest in the spurge family with over 1000 species and is subdivided into many subgenera and sections, a number of which have been treated as distinct genera.[2)] Modern studies have highlighted the widespread use of several of these plants to treat cancerous conditions in the traditional medicine of many areas of the world.[3—6)] The leaves and the latex of this plant are used in the Ayurvedic system of medicine for bronchitis and rheumatism.[7)] Several triterpenes have previously been reported from this plant[8—10)] and we have reported two new ingol derivatives from the latex of this plant.[11)] In continuation of our research interest on these compounds, we reinvestigated the latex of the *Euphorbia nivulia* and isolated three new ingoles, 3-acetyl-8-methoxy-7-angolyl-12-hydroxyingol (**7**), 3,12-diacetyl-7-hydroxy-8-methoxyingol (**8**), and 3,12-diacetyl-7-angolyl-8-hydroxyingol (**9**) apart from known diterpenoids 3,12-diacetyl-7-angeloyl-8-methoxyingol (**2**),[11,12)] 7-angeloyl-12-acetyl-8-methoxyingol (**3**),[11,13)] 3,7,12-triacetyl-8-benzoylingol (**4**),[11,12,14)] 3,12-diacetyl-8-benzoylingol (**5**),[11)] and 3,12-diacetyl-7-benzoyl-8-nicotinylingol (**6**).[11)] All isolated compounds were tested for cytotoxic activity. The structures of the new compounds were deduced by the study of spectral data (UV, IR, ^{1}H-, ^{13}C-NMR, ^{1}H–^{1}H correlation spectroscopy (COSY), and nuclear Overhauser effect spectroscopy (NOESY)) and they were found to be esters of macrocyclic diterpene ingol corresponding to those isolated from other species of the genus. All these compounds were identified as ingol esters bearing various acyl groups such as acetyl, benzoyl, nicotinyl, and angeloyl moieties. The configurations at stereogenic centers were established from coupling constant values, NOE measurements, and comparison with literature data. In all cases the values of $J_{2,3}$ (*ca.* 8.4 Hz) and chemical shifts of 1α and 1β indicated that the configuration at C-2 and C-3 was the same as that in true ingol derivatives.[15)]

Results and Discussion

Compound **7** was obtained as a semisolid, $[\alpha]_D$ −1° (c=0.25, $CHCl_3$), and analyzed for $C_{28}H_{40}O_8$ by high resolution electron impact (HR-EI)-MS (504.2730) and EI mass m/z 504 $[M]^+$ which requires nine degrees of unsaturation. The IR spectrum of compound **7** showed bands at 3300, 1760, and 1720 cm^{-1}, indicating the presence of hydroxyl, keto, and ester carbonyl groups.

The ^{1}H-NMR spectrum of compound **7** showed signals for the presence of an acetate group [δ 2.08 (3H, s)], an angelate group [δ 6.10 (1H, q, J=7.4, 1.5 Hz), 1.93 (3H, d, J=1.5 Hz) and 1.98 (3H, dd, J=7.4, 1.5 Hz)], and a methoxyl group at δ 3.31 (3H, s). Further its ^{1}H-NMR spectrum indicated the presence of an olefinic proton at δ 5.58 (1H, s, H-5), a vinylic methyl at (δ 2.07 (3H, s, H-17), two tertiary methyls at δ 1.11 (3H, s, H-19) and 1.14 (3H, s, H-18), two methine protons geminal to ester functions at δ 5.26 (1H, d, J=8.4 Hz, H-3) and 5.30 (1H, d, J=1.7 Hz, H-7), and a methine proton geminal to ether linkage at δ 2.83 (1H, dd, J=9.9, 1.7 Hz, H-8).

The study of ^{13}C- and distortionless enhancement by polarization transfer (DEPT)-NMR spectra of compound **7** indicated the presence of 28 carbons, which include a ketonic carbonyl group at δ 213.05, two ester carbonyl groups at δ 166.78 and 170.37, and four double bond carbons at δ 117.07, 127.68, 117.07, and 140.42. These spectral data clearly indicate that compound **7** is an ingol derivative[15)] with a methoxyl, an acetate, an angelate, and a secondary hydroxyl group. In its ^{1}H–^{1}H COSY spectrum the signal at δ 0.62 (1H, dd, J=10.7, 9.9 Hz, H-11) showed correlations with δ 3.20 (1H, br t, J=10.7 Hz, H-12) and 1.17 (1H, t, J=9.9 Hz, H-9). Further, the signal at δ 3.20 (1H, br t, J=10.7 Hz, H-12) showed correlation with δ 2.69 (1H, dd, J=7.3, 3.1 Hz, H-13) and the signal at δ 1.17 (1H, t, J=9.9 Hz, H-9) showed correlation with δ 2.83 (1H, dd, J=9.9, 1.7 Hz, H-8). It has



	R_1	R_2	R_3	R_4
2.	Ac	Ang	Me	Ac
3.	H	Ang	Me	Ac
4.	Ac	Ac	Bz	Ac
5.	Ac	H	Bz	Ac
6.	Ac	Bz	Nic	Ac
7.	Ac	Ang	Me	H
8.	Ac	H	Me	Ac
9.	Ac	Ang	H	Ac

Fig. 1

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been shown in the literature[16,20] that the presence of a hydroxyl group at the C-12 position causes the C-11 cyclopropane proton to shift upfield by 0.70 ppm.

From the above correlations, the hydroxyl group was placed at the C-12 position and was further corroborated by NOED experiments. In the NOED experiments, irradiation of the signal at δ 3.20 (1H, br t, J=10.7 Hz, H-12) caused enhancement of the signals at δ 2.83 (1H, dd, J=9.9, 1.7 Hz, H-8), 2.69 (1H, dd, J=7.3, 3.1 Hz, H-13), 2.07 (3H, s, H-17), and 1.11 (3H, s, H-19). The foregoing spectral data and a literature survey[17,20] revealed that the hydroxyl group is present at the C-12 position and the protons at C-7, C-8, C-12, and C-13 are in the β-orientation. Thus the structure of compound **7** was established to be 3-acetyl-8-methoxy-7-angolyl-12-hydroxyingol (**7**).

Compound **8** was obtained as a semisolid, $[\alpha]_D$ +35.0° (c=0.25, $CHCl_3$), analyzed for $C_{25}H_{36}O_8$ by HR-FAB-MS (464.2416) and FAB-MS m/z 464 $[M]^+$ and requires eight degrees of unsaturation. The IR spectrum of compound **8** showed bands at 3300, 2940, 1760, and 1720 cm^{-1}, indicating the presence of hydroxyl, keto carbonyl, and ester carbonyl groups. The ^{1}H-NMR spectrum of compound **8** (Table 1) showed signals for the presence of two acetate groups at δ 2.12 (6H, s) and a methoxyl group at δ 3.36 (3H, s). Further, its ^{1}H-NMR spectrum showed signals for the presence of an olefinic proton at δ 5.77 (1H, s, H-5), a vinylic methyl at δ 1.99 (3H, s, H-17), two tertiary methyls at δ 1.07 (3H, s, H-18) and 0.97 (3H, s, H-19), two methine protons geminal to ester functions at δ 5.20 (1H, d, J=8.4 Hz, H-3) and 4.85 (1H, dd, J=11.1, 4.1 Hz, H-12), a methine proton bearing a hydroxyl group at δ 4.35 (1H, s, H-7), and a methine proton geminal to ether linkage δ 2.86 (1H, d, J=9.7 Hz, H-8).

Examination of the ^{13}C- and DEPT-NMR spectra of compound **8** indicated the presence of 25 carbons, which include a keto carbonyl group at δ 207.64, two ester carbonyl groups at δ 170.70 and 170.47, and a tri-substituted double bond at δ 116.56 (d) and 140.52 (s). From the spectral data, it is observed that one of the proton signal at δ 4.35 (1H, s, H-7) was shifted upfield compared with the methine-bearing ester group, due to the presence of a hydroxyl group. The disposi-

Table 1. NMR Spectral Data of Compounds **7**—**9** (400 MHz, $CDCl_3$)

Position	Compound **7**			Compound **8**			Compound **9**		
	^{1}H-NMR (J in Hz)	^{13}C-NMR	^{1}H–^{1}H-NOESY	^{1}H-NMR (J in Hz)	^{13}C-NMR	^{1}H–^{1}H-NOESY	^{1}H-NMR (J in Hz)	^{13}C-NMR	^{1}H–^{1}H-NOESY
1α	1.69 d (15.1)	31.05	H-1β	1.70 d (14.5)	31.59	H-1β, H-16	1.69 d (14.5)	31.58	H-1β
1β	2.72 dd (13.9, 9.1)		H-1α, H-2	2.81 dd (14.5, 8.0)		H-1α	2.79 q (15.0, 9.0)		H-1α, H-2
2	2.46 m	29.13	H-3, H-16	2.60 m	28.41	H-3, H-16	2.47 m	29.53	H-3, H-1β
3	5.26 d (8.4)	76.50	H-2, H-5	5.20 d (8.4)	77.74	H-2, H-5	5.26 d (8.5)	76.60	H-2, H-5
4	—	73.99	—	—	73.71	—	—	73.49	—
5	5.58 s	117.07	H-3	5.77 s	116.56	H-3	5.54 s	116.74	H-3
6	—	140.42	—	—	140.52	—	—	138.37	—
7	5.30 d (1.7)	74.67	H-8, H-17, H-5, OMe	4.35 s	73.03	H-8, H-13, H-17, OMe	5.10 d (2.0)	70.96	H-8, H-17
8	2.83 dd (9.9, 1.7)	71.68	H-7, H-12, H-17	2.86 d (9.7)	71.34	H-12, H-7	3.54 dd (10.4, 2.0)	69.85	H-7, H-12, H-13, H-16, H-17
9	1.17 t (9.9)	27.22	H-11	1.17 t (9.7)	25.77	—	1.18 t (10.4)	29.53	H-11
10	—	18.74	—	—	18.94	—	—	19.09	—
11	0.62 dd (10.7, 9.9)	34.05	H-18	1.02 dd (11.1, 9.7)	31.59	—	0.99 dd (10.4, 10.8)	31.25	H-9
12	3.20 br t (10.7)	71.68	H-8, H-13, H-17, H-19	4.85 dd (11.1, 4.1)	80.76	H-8, H-13, H-19	4.96 dd (10.8, 4.0)	80.20	H-8, H-13, H-16, H-17
13	2.69 dd (7.3, 3.1)	43.05	H-7, H-12	2.93 m	43.03	H-7, H-12	2.94 m	43.09	H-8, H-12, H-16, H-17
14	—	213.05	—	—	207.64	—	—	207.50	—
15	—	70.80	—	—	71.00	—	—	71.03	—
16	0.95 d (7.4)	17.79	—	0.90 d (7.5)	16.56	—	0.95 d (7.4)	16.58	H-8, H-12, H-13
17	2.07 s	20.51	—	1.99 s	17.63	—	2.09 s	17.78	
18	1.14 s	29.51	—	1.07 s	29.07	—	1.09 s	29.23	—
19	1.11 s	16.92	—	0.97 s	16.88	—	1.09 s	16.90	—
20	1.26 d (7.4)	14.59	—	1.05 d (6.8)	13.22	—	1.05 d (7.4)	13.35	—
OAc	2.08 s	15.97, 170.37	—	2.12 s	20.99, 170.70	—	2.06 s	20.97, 170.55	—
				2.12 s	20.56, 170.47		2.11 s	20.48, 170.24	
OMe	3.31 s	56.24	—	3.36 s	56.45	—	—	—	—
			—						
OH	—	—	—	2.45 s	—	—	—	—	—
			—						
OAng	6.10 q (7.4, 1.5)	117.07, 20.40		—			6.1 dd (7.4, 1.4)	138.38, 20.34	—
	1.93 d (1.5)	15.74					1.94 d (1.4)	15.75	
	1.98 dd (7.4, 1.5)	127.68, 166.78					1.98 dd (7.4, 1.4)	127.72, 166.94	

tion of the hydroxyl group and stereochemistry of various functional groups were deduced by ^{1}H–^{1}H-COSY, NOESY, and NOED experiments. In the ^{1}H–^{1}H COSY spectrum, the signal at δ 5.20 (1H, d, J=8.4 Hz, H-3) showed correlation with δ 2.60 (1H, m, H-2). The signal at δ 4.85 (1H, dd, J=11.1, 4.1 Hz, H-12) showed correlations with δ 2.93 (1H, m, H-13) and 1.02 (1H, dd, J=11.1, 9.7 Hz, H-11). The signal at δ 2.86 (1H, d, J=9.7 Hz, H-8) showed correlation with δ 1.17 (1H, t, J=9.7 Hz, H-9). In the NOESY spectrum, the signal at δ 4.35 (1H, s, H-7) showed correlations with δ 2.86 (1H, d, J=9.7 Hz, H-8), 2.93 (1H, m, H-13), and 1.99 (3H, s, H-17). The signal at δ 4.85 (1H, dd, J=11.1, 4.1 Hz, H-12) showed correlations with δ 2.86 (1H, d, J=9.7 Hz, H-8), 2.93 (1H, m, H-13), and 0.97 (3H, s, H-19). The spectral data indicate that the hydroxyl group is situated at C-7 and the substituents at C-7, C-8, C-12, and C-13 are in the α-orientation. This was further confirmed by NOED experiments. In the NOED experiments, irradiation of the signal at δ 4.85 (1H, dd, J=11.1, 4.1 Hz, H-12) caused enhancement of the signals at δ 2.86 (1H, d, J=9.7 Hz, H-8), 2.93 (1H, m, H-13), and 0.97 (3H, s, H-19). Irradiation of the signal at δ 4.35 (1H, s, H-7) caused enhancement of signals at δ 2.86 (1H, d, J=9.7 Hz, H-8), 1.99 (3H, s, H-17), 3.36 (3H, s, OMe), 5.77 (1H, s, H-5), and 2.45 (1H, s, OH). These experiments further confirmed that the hydroxyl group is located at C-7 and that the C-7, C-8, C-12 and C-13 protons are in the β-orientation. Irradiation of the signal at δ 5.20 (1H, d, J=8.4 Hz H-3) caused enhancement of the signals at δ 5.77 (1H, s, H-5) and 2.60 (1H, m, H-2). Irradiation of the signal at δ 5.77 (1H, s, H-5) caused enhancement of the signals at δ 1.17 (1H, t, J=9.7 Hz, H-9) and 5.20 (1H, d, J=8.4 Hz, H-3). The above spectral data indicate that the protons at the C-2 and C-3 positions are in the α-orientation. Thus the structure of compound **8** was unambiguously established to be 3,12-diacetyl-7-hydroxy-8-methoxyingol (**8**).

Compound **9** was obtained as a semisolid, $[\alpha]_D$ −3.6° (c=0.125, $CHCl_3$), analyzed for $C_{29}H_{40}O_9$ by HR-FAB-MS (532.2680) and its FAB mass showed a molecular ion peak at m/z 555 $[M+Na]^+$. The IR spectrum of compound **9** showed bands at 3340, 1760, and 1710 cm^{-1}, indicating the presence of hydroxyl, keto carbonyl, and ester carbonyl groups. The ^{1}H-NMR spectrum revealed the presence of two acetates at δ 2.06 (3H, s) and 2.11 (3H, s) and an angelate group [δ 6.1 (1H, dd, J=7.4, 1.4 Hz), 1.94 (3H, d, J=1.4 Hz), and 1.98 (3H, dd, J=7.4, 1.4 Hz)]. Further, its ^{1}H-NMR spectrum indicated the presence of an olefinic proton at δ 5.54 (1H, s, H-5), a vinylic methyl at δ 2.09 (3H, s, H-17), two tertiary methyls as singlets at δ 1.09 (6H, s, H-18 and H-19), three methine protons geminal to ester functions at δ 5.26 (1H, d, J=8.5 Hz, H-3), 5.10 (1H, d, J=2.0 Hz, H-7), and 4.96 (1H, dd, J=10.8, 4.0 Hz, H-12), and a methine proton bearing an oxygen atom at δ 3.54 (1H, dd, J=10.4, 2.0 Hz, H-8).

The ^{13}C-NMR spectrum displayed signals for 29 carbons, which include a ketonic carbonyl at δ 207.50, three ester carbonyls at δ 170.55, 170.24, and 166.94, four double-bond carbons at δ 138.38, 127.72, 116.74, and 138.37, and six oxygen-bearing carbons at δ 74.49, 71.03, 76.60, 70.96, 80.20, and 69.85. The above spectral data reveal that compound **9** is an ingol derivative. In the ^{1}H–^{1}H COSY spectrum, the signal at δ 5.26 (1H, d, J=8.5 Hz, H-3) showed correlation with δ 2.47 (1H, m, H-2). The signal at δ 3.54 (1H, dd, J=10.4, 2.0 Hz, H-8) showed correlations with a methine-bearing angelate group at δ 5.10 (1H, d, J=2.0 Hz, H-7) and with a cyclopropane methine at δ 1.18 (1H, t, J=10.4 Hz, H-9). The signal at δ 4.96 (1H, dd, J=10.8, 4.0 Hz, H-12) showed correlations with δ 0.99 (1H, dd, J=10.4, 10.8 Hz, H-11) and δ 2.94 (1H, m, H-13). The spectral data indicate that the hydroxyl group is at the C-8 position. In its NOESY spectrum, the signal at δ 3.54 (1H, dd, J=10.4, 2.0 Hz, H-8) showed correlations with δ 5.10 (1H, d, J=2.0 Hz, H-7), 4.96 (1H, dd, J=10.8, 4.0 Hz, H-12), 2.94 (1H, m, H-13), 0.95 (3H, d, J=7.4 Hz, H-16), and 2.09 (3H, s, H-17). The above spectral data imply that the protons at C-7, C-8, C-12, and C-13 are in the β-orientation. Thus the structure of compound **9** is confirmed to be 3,12-diacetyl-7-angolyl-8-hydroxyingol (**9**).

Previously we reported that when compounds **2**—**6** were examined for prostaglandin E_2 (PGE_2) inhibitory activity, only isolate **3** showed significant inhibition (IC_{50} 0.003 μM).[11] As these diterpenes are known for their diverse biological activities,[18—20] we tested compounds **2**—**8** for their potential anticancer activity. Of these, compounds **2**, **3**, and **8** showed significant cytotoxic activity (Table 2) against Colo 205, MT2, and CEM cell lines and others showed moderate or no activity. As the LD_{50} values are almost same in the three cell lines for compounds **2**, **3**, and **8**, it is worth investigating their *in vitro* inhibition of the proliferation of other types of cancer cells.

Table 2. Cytotoxic Activity of Compounds Isolated from *E. nivulia*

Compound	Colo 205 (LD_{50}, μM)[a]	MT2 (LD_{50}, μM)[a]	CEM (LD_{50}, μM)[a]
2	13.92	13.92	13.92
3	22.85	22.85	22.85
8	16.81	14.44	13.58

a) LD_{50}, lethal dose.

Experimental

General The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity INOVA 500 MHz spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively, and all 1D and 2D spectra (^{1}H–^{1}H COSY, NOESY, NOED) were recorded using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million, and coupling constants (J) are expressed in Hz. Silica gel (100—200 mesh, ACME) was used for column chromatography. UV and IR spectra were recorded on Shimadzu 240 and Perkin-Elmer RXI FT-IR spectrophotometers, respectively. Mass spectra were recorded on a Finnigan-MAT 1020 instrument. Optical rotations were measured on a JASCO DIP-370 polarimeter.

Plant Material Collection and Identification The latex of the plant material of *E. nivulia* (Euphorbiaceae) was collected from Bhadrachalam forest, Andhra Pradesh, India, in January 2000 and identified by Professor M. Prabhakar Rao, Department of Botany, Osmania University, Hyderabad, India. A voucher specimen (No. 001662) was deposited in the herbarium of the Department of Botany, Osmania University.

Extraction and Fractionation Procedure The frozen latex of *E. nivulia* was dried under a vacuum drier at 35 °C for 24 h to obtain a white powder (750 g) and extracted with methanol (3×1.5 l) at room temperature. The combined methanol extract was filtered and evaporated under reduced pressure to yield a brown gummy residue (58 g), which was subjected to silica gel (100—200 mesh) chromatography eluted with hexane through hexane/ethyl acetate mixtures to ethyl acetate to give four fractions. Fraction A was further separated by column chromatography (hexane–acetone, 95 : 5) to afford compound **1** (14.5 g); fraction B was further separated by column chromatography (hexane–acetone, 90 : 10) to afford compounds **2** (2.4 g), **3** (30 mg), **4** (100 mg), and **7** (70 mg); fraction C was further separated by column chromatography (hexane–acetone, 80 : 20) to afford compounds **5** (150 mg),

6 (40 mg), and **8** (200 mg); and fraction D was further separated by column chromatography (hexane–acetone, 75 : 25) to afford compound **9** (15 mg).

3-Acetyl-8-methoxy-7-angeloylingol (**7**): Obtained as a semisolid; $[\alpha]_D^{25}$ −1.0° (c=0.25, $CHCl_3$); IR (KBr) ν_{max} 3300, 1760, 1720 cm^{-1}; ^{1}H-NMR data, see Table 1; ^{13}C-NMR data, see Table 1; HR-EI-MS, observed m/z 504.2730 $C_{28}H_{40}O_8$; requires m/z 504.2723 [M^+]. EI-MS observed m/z (%): 505 $[M+1]^+$ (15), 472 (16), 392 (15), 250 (35), 221 (19), 165 (25), 122 (21), 105 (61).

3,12-Diacetyl-7-hydroxy-8-methoxyingol (**8**): Obtained as a semisolid; $[\alpha]_D^{25}$ +35.0° (c=0.25, $CHCl_3$); IR (KBr) ν_{max} 3300, 2940, 1760, 1720 cm^{-1}; ^{1}H-NMR data, see Table 1; ^{13}C-NMR data, see Table 1; HR-FAB-MS, observed m/z 464.2416 $C_{25}H_{36}O_8$; requires m/z 464.2410 $[M]^+$. FAB-MS m/z (%): 464 $[M]^+$ (6), 433 (27), 404 (11), 313 (5), 267 (4), 109 (45), 83 (34), 55 (27).

3,12-Diacetyl-7-angeloyl-8-hydroxyingol (**9**): Obtained as a semisolid; $[\alpha]_D^{25}$ −3.6° (c=0.125, $CHCl_3$); IR (KBr) ν_{max} 3340, 1760, 1710 cm^{-1}; ^{1}H-NMR data, see Table 1; ^{13}C-NMR data, see Table 1; HR-FAB-MS, observed m/z 532.2680 $C_{29}H_{40}O_9$; requires m/z 532.2672 $[M]^+$. FAB-MS observed m/z (%): 555 $[M+Na]^+$ (5), 531 (8), 515 (28), 473 (3), 433 (3), 307 (30), 154 (100), 137 (75), 107 (31), 83 (80), 69 (29), and 55 (49).

Cytotoxic Activity The compounds were tested for their antiproliferative activity using the methylthioazotetrazolium (MTT) colorimetric assay.[21] The assay was performed in a flat-bottomed 96-well tissue culture plate. The Colo 205, MT2, and CEM cell lines were cultured in RPMI 1640 (Sigma cat # R4130) with 10% FBS (Sigma cat # F2442). One hundred microliters of medium containing 5×10^4 cells were cultured with increasing concentrations of the compounds and incubated for 24 h at 37 °C in an incubator with a 5% CO_2 atmosphere. MTT 20 μl (Sigma cat # M5655) was added to the culture to achieve a final concentration of 0.5—1 mg/ml and incubation continued for 4 h at 37 °C under 5% CO_2 atmosphere. A purple-blue formazan precipitate forms upon reduction of MTT by mitochondria of proliferating cells. If cells are nonviable, the culture medium remains yellow. The precipitate was dissolved by incubation with acidic isopropanol 100 μl for 30 min. The intensity of color developed was measured at 490 nm. Etoposide was used as the standard inhibitor. The percentage of viability was calculated based on the results of a control experiment conducted in the absence of an inhibitor.

The LD_{50} was determined based on the concentration required for inhibiting 50% viability of a given cell line. The data represent an average of three independent determinations.

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