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SYNTHESIS AND CYTOTOXICITY OF DERIVATIVES OF DIPTEROCARPOL, A METABOLITE OF *Dipterocarpus alatus*

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New derivatives with hydroxy, epoxy, hydroxyimino, acetoxy, lactol, methoxylactol, and indole groups in ring A and the side chain were synthesized via chemical transformations of dipterocarpol. The structure–cytotoxicity relationship was described for the dipterocarpol derivatives.

Keywords: dammarane triterpenoids, dipterocarpol, synthesis, oxidation, structure–cytotoxicity.

Dipterocarpol [**1**, 20(*S*)-dammar-24-en-3-one)], a dammarane-type triterpenoid, and its derivatives exhibit various types of activity (anticancer [1], antiviral [2–4], immunostimulating [5], etc.). Recent studies have found antitumor activity of dammarane metabolites from *Panax ginseng* root with IC_{50} from 4 to 8 μM against HepG2, Colon205, and HL-60 cells [6, 7]. Metabolites of fruit and leaves of *Aglaia erythrosperma* exhibited cytotoxicity with IC_{50} from 5 to 8 μM against epidermoid carcinoma KB, breast cancer BC, and lung cancer NCI-H187 cells [8]. Conjugates of dammaranic acid with various L-amino acids were active against melanoma CRL1579 (EC_{50} 7.5–14.5 μM) and leukemia HL60 (EC_{50} 4.7–21.7 μM) cells [9]. Synthetically produced 20(*S*)-20-hydroxy-3,4-seco-dammara-4(28),24-dien-3-al inhibited carcinogenesis (skin cancer) in mice [9]. The cytotoxicity of dammarane triterpenoids depends to a large extent on the number and location of hydroxyls in their structures. For example, 7β-hydroxydipterocarpol exhibited cytotoxicity against HeLa and COS-1 cells (IC_{50} 100 and 200 μM) whereas 7β,11α-dihydroxydipterocarpol was inactive [10]. The aglycons of ginseng root saponins 20(*S*)-protopanaxadiol and 20(*S*)-protopanaxatriol are close structural analogs of **1** that were approved as the preparation Pandimex in China for treating metastatic cancer of the lung, breast, spleen, stomach, and rectum. These dammarane saponins cause apoptosis of cells and inhibit P-glycoprotein [11]. Thus, chemical modification of **1** and the study of the cytotoxicity of its derivatives are highly critical.

Herein we present results on a series of new transformations of **1** that was isolated from sap of the tree *Dipterocarpus alatus* growing in Vietnam.

Oxidation of **1** with ozone in CH_2Cl_2 without reduction of the peroxide products produced *tris*-*nor*-lactol **2** (Scheme 1). Characteristic resonances of C-20 and C-24 were observed in the ^{13}C NMR spectrum at δ 87.6 and 99.7 ppm. A broad resonance for the C-24 O<u>H</u> proton at δ 4.51 ppm was characteristic of the PMR spectrum of **2**. Ozonolysis of **1** in MeOH formed methoxylactol **3** in 75% yield as a 1:1 mixture of racemates at the C-24 position. The structure of **3** was inferred from the doubled resonances of C-24 of equal intensity at δ 103.1 ppm and of the OCH_3 protons at δ 3.33 and 3.34 ppm.

We also used *m*-chloroperbenzoic acid (*m*-CPBA), dimethyldioxirane, and OsO_4 as oxidants of **1**. Both *m*-CPBA and dimethyldioxirane epoxidized the C-24(25) double bond to form a mixture of stereoisomeric epoxides **6** (ratio of α:β isomers 0.6:0.4 and 0.7:0.3, respectively) according to PMR spectra. Oxidation of **1** by OsO_4 in aqueous THF produced 24,25-diol **5**.

Reduction of **1** by $NaBH_4$ in MeOH occurred with formation of 3β-hydroxydipterocarpol (**4**). The action of $NaBH_4$ on 24,25-epoxide **6** produced a mixture of the oxirane ring-opening product 20,25-diol **7** and 3β-hydroxy-24,25-epoxide **8**. These were separated by column chromatography. The spectrum of **8** showed resonances for the epoxy group and for the 3-hydroxy group at δ 78.9 ppm. The spectrum of **7** contained resonances for C-3 and C-24 at δ 78.94 and 75.48 ppm. Acylation of **8** by Ac_2O in Py gave acetate **9** (Scheme 1).

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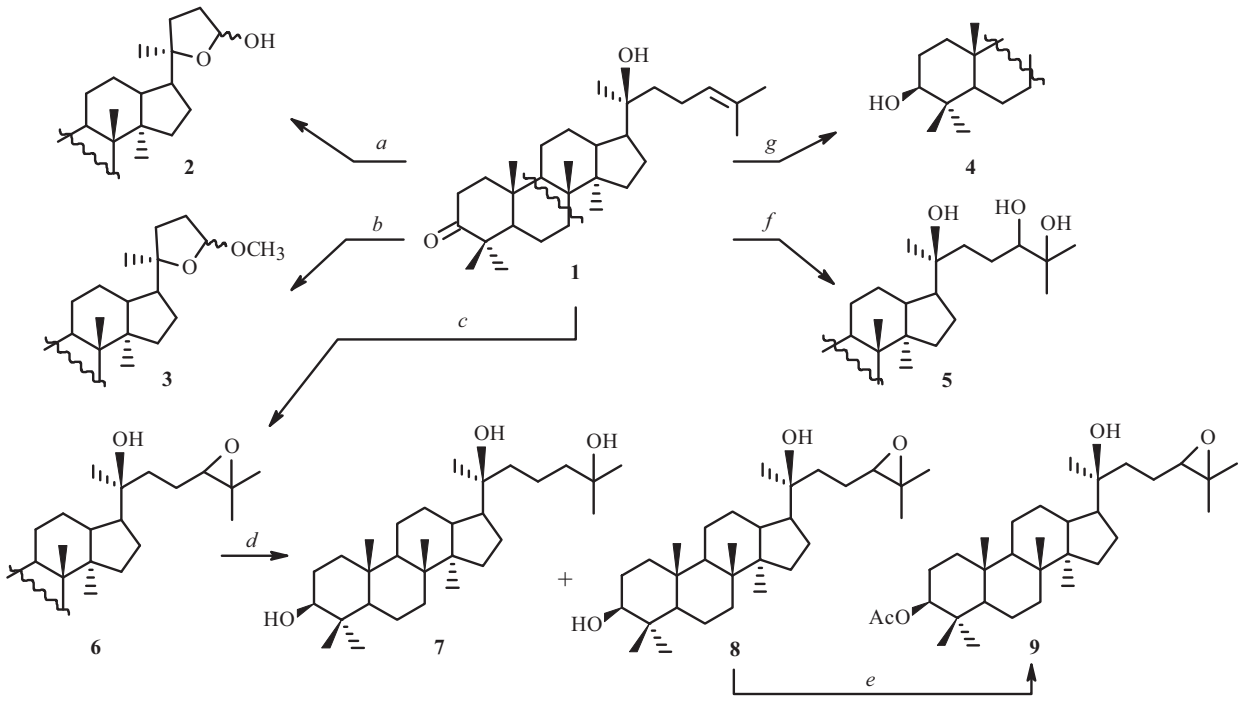
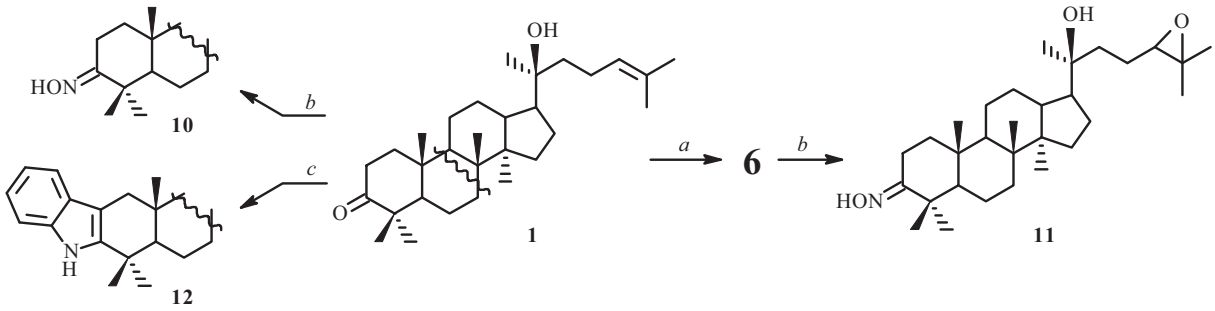


a. 1 eq. O_3, CH_2Cl_2, –40°C; *b*. 2 eq. O_3, MeOH, –40°C; *c*. *m*-CPBA/$CHCl_3$ or dimethyldioxirane:Me_2CO; *d*. $NaBH_4$, CH_2Cl_2, 0–5°C; *e*. Ac_2O, Py, DMAP; *f*. OsO_4, NMO, THF:H_2O 10:1; *g*. $NaBH_4$, EtOH, reflux.

Scheme 1



a. *m*-CPBA, CH_2Cl_2; *b*. $NH_2OH{\cdot}HCl$, DMAP, Py:MeOH 1:1; *c*. $PhNHNH_2$, AcOH, 100°C.

Scheme 2

Reaction of **1** and its 24,25-epoxide **6** with hydroxylamine hydrochloride synthesized ketoximes **10** and **11** as two isomers at the C-24(25) position according to NMR spectra (Scheme 2). Reaction of **1** with phenylhydrazine in AcOH (Fisher reaction) gave 2,3-indole **12** in 86% yield. Resonances for C-2 and C-3 at δ 140.9 and 110.3 ppm and for the aromatic substituent at δ 107.0–136.2 ppm indicated that **12** had formed. The PMR spectrum showed resonances for the aromatic protons at δ 7.02–7.81 ppm.

The antitumor activity (cytotoxicity) of dipterocarpol derivatives **1**–**4**, **6**, **7**, and **12** was studied *in vitro* in 60 cell lines of nine different human tumors (lung, colon, CNS, ovary, kidney, prostate, brain, leukemia, melanoma) using the method described by the National Cancer Institute of the USA (NCI) [12-15]. Compounds were placed in cell culture medium at concentration 10^{-5} M for 48 h for preliminary testing. Then, growth of treated cells was compared with that of untreated control cells. Table 1 presents the results in percent growth of treated cells compared with control cells (negative values correspond to cell death). According to the criterion adopted by the NCI, compounds are considered active if they inhibit cell growth to 32% of the control or cause their death. The investigated dipterocarpol derivatives did not exhibit antitumor activity *in vitro* against the studied cells.

TABLE 1. Antitumor Activity of **1**–**4**, **6**, **7**, and **12** for 60 Human Tumor Cell Lines at *in vitro* Concentration 10^{-5} M

Cell line	**1**	**2**	**3**	**4**	**6**	**7**	**12**
NSC lung cancer					101.67		
A549/ATCC	71.49	103.43	101.61	102.58	105.91	85.17	100.77
EKVX	69.80	105.25	103.93	85.83	96.64	99.67	80.91
HOP-62	100.03	113.97	115.31	101.33	–	91.63	105.14
NCI-H226	93.93	123.54	107.53	102.88	–	102.57	103.60
NCI-H322M	–	–	–	–	100.02	–	–
NCI-H23	62.23	123.54	100.81	100.43	107.68	99.70	97.22
NCI-H460	70.33	101.58	109.18	109.10	89.07	118.17	101.98
NCI-H522	89.90	108.86	96.85	103.89	96.70	87.25	118.31
HOP-92	68.35	95.34	83.95	105.13		101.71	90.72
Colon cancer					115.79		
COLO 205	90.72	117.59	116.52	113.63	104.69	108.21	107.10
HCC-2998	76.80	102.69	108.64	113.33	95.37	101.03	102.92
HCT-116	48.88	98.25	93.77	118.97	102.91	114.12	97.86
HCT-15	74.43	103.36	91.92	116.04	95.33	105.69	114.27
HT29	52.14	100.61	96.89	105.39	97.01	94.63	109.76
KM12	85.83	105.38	99.59	118.71	116.74	105.58	112.56
SW-620	76.32	112.38	112.17	109.00		103.25	109.39
Breast cancer					98.60		
BT-549	97.65	96.20	94.02	129.84	106.69	128.16	112.13
HS 578T	88.28	108.79	111.87	111.01	96.61	–	101.15
MCF7	60.79	98.42	94.31	88.68	120.10	88.61	86.07
MDA-MB-231/ATCC	94.50	124.43	115.23	108.94	–	99.83	99.97
MDA-MB-435	–	–	–	–	111.82	–	–
MDA-MB-468	88.73	104.43	103.25	104.30	–	86.83	115.18
NCI/ADR-RES	–	–	–	–	87.99	–	–
T-47D	81.81	97.04	99.95	90.38		95.83	79.85
Ovarian cancer					109.81		
IGROV1	110.93	112.95	103.33	125.45	112.92	114.87	112.28
OVCAR-3	112.03	121.23	112.76	127.08	–	100.96	122.15
OVCAR-4	–	–	–	–	108.69	108.38	–
OVCAR-5	97.69	104.86	109.06	125.77	106.74	108.60	117.22
OVCAR-8	91.90	104.08	102.23	105.10	105.37	98.49	102.07
SK-OV-3	102.78	107.01	104.07	118.12	106.37	95.44	111.08
NC/ADR-RES	91.47	98.80	97.92	102.73		105.35	94.85
Leukemia					–		
CCRF-CEM	–	-	–	–	95.83	102.68	–
HL-60(TB)	46.32	96.61	93.94	109.16	101.90	120.56	70.49
K-562	38.60	96.76	84.86	96.19	95.05	109.63	88.06
MOLT-4	51.02	97.09	76.36	105.64	98.37	99.99	113.28
RPMI-8226	31.97	100.16	82.81	92.16	95.30	100.68	80.88
SR	45.38	90.29	76.45	92.36		92.46	103.15
Renal cancer					95.96		
786-0	65.79	99.78	98.11	113.67	107.37	114.15	97.09
A498	75.78	98.07		102.43	105.60	–	89.18
ACHN	114.11	111.80	112.23	119.25	–	106.70	98.62
CAKI-1	–	101.64	102.88	92.97	99.08	99.26	87.16
RXF 393	83.65	109.68	117.93	106.30	125.61	113.66	102.61
SN12C	86.78	106.90	108.34	104.05	104.31	92.11	99.99
TK-10	112.19	107.55	112.11	125.42	120.19	88.92	133.56
UO-31	75.57	99.43	102.25	109.50	98.57	97.68	96.14
Melanoma							
LOX IMVI	90.25	101.92	105.01	102.89	107.29	97.43	95.15
M14	73.88	103.39	107.86	120.92	100.32	116.52	106.98
MALME-3M	98.72	100.90	111.57	125.31	92.29	103.38	112.95
SK-MEL-2	105.11	93.16	100.59	122.34	90.53	104.13	131.63

TABLE 1. (continued)

Cell line	1	2	3	4	6	7	12
SK-MEL-28	102.18	115.52	112.29	108.97	112.57	99.95	103.44
SK-MEL-5	99.50	106.62	97.63	101.20	110.57	98.52	105.28
UACC-257	110.93	105.96	109.37	109.24	112.75	96.40	116.50
UACC-62	87.47	102.45	98.11	108.14	101.95	97.43	102.87
MDA-MB-435	64.19	100.57	100.42	95.84	98.05	–	96.68
Prostate cancer							
DU-145	48.78	120.28	109.20	121.77	114.98	110.38	122.33
PC-3	68.60	91.64	75.04	92.77	96.88	91.68	84.11
CNS cancer							
SF-268	95.24	109.71	107.13	122.97	99.46	101.91	123.35
SF-295	–	104.46	90.58	102.40	91.76	105.60	89.72
SF-539	93.10	104.64	95.61	105.75	111.04	104.16	107.63
SNB-19	100.73	111.27	118.74	110.59	112.61	98.71	100.30
SNB-75	81.64	100.04	108.12	97.78	98.85	93.64	92.25
U251	85.56	102.90	98.54	97.27	103.37	96.21	102.61

The activity of compounds **1**, **5**–**9**, and **11** were tested at the Institute of Chemistry, Vietnamese Academy of Science and Technology (IC VAST), for human epidermoid cancer KB tumor cells. The data are presented below:

Compound	*IC_{50}, μg/mL*	*Compound*	*IC_{50}, μg/mL*
1	14.37	**8**	10.70
5	> 128	**9**	> 128
6	> 128	**11**	> 128
7	> 128	Ellipticine	0.51.

It was found for **5**–**7**, **9**, and **11** that IC_{50} was >128 μg/mL, i.e., substituents such as indole and oxime in ring A and lactol, epoxide, and 24,25-diol in the side chain decreased the cytotoxicity of **1**. The IC_{50} values for **1** and its derivative **8** were 14.37 and 10.07 μg/mL. According to the selected activity criterion (IC_{50} < 4 μg/mL [16]), these compounds exhibited weak cytotoxicity. Introducing 3β-hydroxy and C-24,25-epoxide groups (**8**) into the structure of **1** enhanced insignificantly the cytotoxicity. The simultaneous presence of three hydroxyls in the C-3, C-20, and C-25 positions (**7**) did not affect the activity although literature data [10] indicated that several such groups were important for enhancing the activity.

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance III pulsed spectrometer (500.13 and 125.47 MHz, respectively) using a 5-mm probe with *Z*-gradient PABBO at constant sample temperature 298 K. Chemical shifts in NMR spectra are given in ppm relative to $SiMe_4$ internal standard. Mass spectra were obtained on an Agilent 6310 instrument. Melting points were determined on a Boetius microstage. Specific rotation angles were taken on a Perkin-Elmer 241 MC polarimeter. Elemental analyses were performed on an EuruEA-3000 CHNS-analyzer using acetanilide as a primary standard. We used an Ozon-4K ozonator (Russia). TLC was carried out on Sorbfil plates (ZAO Sorbpolimer, Russia) using $CHCl_3$:EtOAc (20:1). Compounds were detected by H_2SO_4 solution (10%) with subsequent heating at 100–120°C for 2–3 min. Compound **1** was isolated as before [17].

24(*R*,*S*)-Hydroxy-25,26,27-*tris*-*nor*-20(*S*)-dammar-3-one (2). Ozone (1 eq.) was passed through a solution of **1** (1 mmol, 0.44 g) in CH_2Cl_2 (50 mL) at –40°C until the starting material disappeared (TLC monitoring). The solvent was evaporated. The product was chromatographed over a column of Al_2O_3 with elution successively by C_6H_6 and $CHCl_3$. Yield 0.33 g (75%) as a 1:1 mixture of two racemates (according to PMR spectra), mp 208–210°C, $[\alpha]_D^{20}$ +106° (*c* 0.25, $CHCl_3$). $C_{27}H_{43}O_3$. PMR spectrum (δ, ppm): 0.83 (3H × 0.5, s, CH_3), 0.84 (3H × 0.5, s, CH_3), 0.91 (3H, s, CH_3), 0.96 (3H × 0.5, s, CH_3), 0.98 (3H × 0.5, s, CH_3), 0.97 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.08 (3H, c, CH_3), 1.15–1.65 (13H, m, CH, CH_2), 1.67–2.11 (7H, m, CH, CH_2), 2.35–2.59 (3H, m, CH, CH_2), 3.32 (1H × 0.5, m, OH), 3.68 (1H × 0.5, m, OH), 4.96 (1H, br.s, H-23). ^{13}C NMR spectrum (δ, ppm): 15.2, 16.2, 19.6, 22.1, 24.3, 25.4, 26.7, 27.1, 28.8, 31.1, 31.7, 32.4, 32.8, 33.8, 34.1, 34.6, 36.8,

40.3, 43.5, 47.4, 49.7, 50.0, 55.3, 62.2 (0.5 × C), 61.9 (0.5 × C), 87.9 (0.5 × C-20), 87.6 (C-20),103.6 (0.5 × C-24), 103.1 (0.5 × C-24), 217.9 (C-3).

24(*R*,*S*)-Methoxy-25,26,27-*tris*-*nor*-20(*S*)-dammar-3-one (3). Ozone (2 eq.) was passed through a solution of **1** (1 mmol, 0.44 g) in MeOH (50 mL) at –40°C until the starting material disappeared (TLC monitoring). The solvent was evaporated. The product was chromatographed over a column of Al_2O_3 with elution successively by C_6H_6 and $CHCl_3$. Yield 0.30 g (70%) as a 1:1 mixture of two racemates (according to PMR spectra), mp 142–145°C, $[\alpha]_D^{20}$ +36° (*c* 0.25, $CHCl_3$). $C_{28}H_{46}O_3$. PMR spectrum (δ, ppm, J/Hz): 0.90 (3H, s, CH_3), 0.94 (3H, c, CH_3), 0.98 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.11–1.64 (13H, m, CH, CH_2), 1.70–2.19 (8H, m, CH, CH_2), 2.46–2.75 (3H, m, CH, CH_2), 3.33 and 3.34 (3H, s, OCH_3, ratio 1:1), 4.93 (1H, t, J = 4.7, H-24). ^{13}C NMR spectrum (δ, ppm): 15.2, 16.0, 16.1, 19.6, 21.0, 21.9, 25.0, 25.5, 26.7, 26.8, 29.1, 31.1, 31.2, 34.0, 34.5, 36.8, 39.8, 40.3, 41.0, 43.3, 47.4, 49.3, 50.0, 50.1, 55.3, 88.1 and 87.9 (C-20), 104.7 and 105.2 (C-24), 217.9 (C-3).

20(*S*)-Hydroxydammar-24-en-3β-ol (4). A solution of **1** (1 mmol, 0.44 g) in EtOH (30 mL) was treated with $NaBH_4$ (2.5 mmol, 0.1 g), refluxed for 4 h, and poured into HCl solution (20 mL, 5%). The precipitate was filtered off, washed with H_2O, dried, and purified by column chromatography over Al_2O_3 using $CHCl_3$ eluent. Yield 0.42 g (96%), mp 134–135°C, $[\alpha]_D^{20}$ +20° (*c* 1.3, $CHCl_3$) (lit. [18] mp 133–135°C). $C_{30}H_{52}O_2$. PMR spectrum (δ, ppm , J/Hz): 0.69 (3H, s, CH_3), 0.76 (3H, s, CH_3), 0.79 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.89 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.10–1.29 (7H, m, CH, CH_2), 1.31–1.49 (14H, m, CH, CH_2), 1.63–1.77 (7H, m, CH, CH_2), 1.91–2.30 (3H, m, CH, CH_2), 3.12 (1H, dt, J_1 = 5.3, J_2 = 5.4, J_3 = 10.7, CH), 4.21 (1H, br.s, OH), 5.12 (1H, t, J = 6.9, CH). ^{13}C NMR spectrum (δ, ppm): 15.4, 15.5, 16.2, 16.5, 17.7, 18.3, 21.6, 22.6, 24.8, 25.4, 25.7, 27.4, 27.6, 28.0, 31.2, 35.3, 37.1, 39.0, 39.1, 40.4, 40.5, 42.3, 49.9, 50.3, 50.7, 55.9, 75.34 (C-20), 78.94 (C-3), 124.68 (C-24), 131.63 (C-25).

20(*S*)-Hydroxydammar-24,25-diol-3-one (5). A solution of **1** (1 mmol, 0.44 g) in THF:H_2O (10:1) was treated with *N*-methylmorpholine-*N*-oxide (NMO, 1.5 mmol) and OsO_4 (0.02 mol), stirred at room temperature for 4 h, and treated with $NaHSO_3$ solution (3 mL, 20%). The layers were separated. The aqueous layer was extracted with EtOAc (4 × 4 mL). The combined organic layers were washed until neutral and dried over Na_2SO_4. The solvent was evaporated in vacuo. The solid was purified by column chromatography over SiO_2 using CH_2Cl_2:MeOH (100:1) eluent. Yield 0.34 g (78%), mp 139°C, $[\alpha]_D^{20}$ –47° (c 1.0, $CHCl_3$). $C_{30}H_{52}O_4$. PMR spectrum (δ, ppm , J/Hz): 0.88 (3H, s, CH_3), 0.94 (3H, s, CH_3), 1.00 (3H, d, J = 0.04, CH_3), 1.04 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.16 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.23 (3H, d, J = 0.01, CH_3), 1.24–1.67 (14H, m, CH, CH_2), 1.68–1.95 (6H, m, CH, CH_2), 2.39–2.51 (4H, m, CH), 3.40 (1H, dd, J_1 = 10.4, J_2 = 2.7, H-24), 4.23 (3H, br.s, 3-OH). ^{13}C NMR spectrum (δ, ppm): 15.2, 16.0, 16.4, 16.5, 19.7, 21.0, 22.0, 24.9, 25.1, 25.4, 25.4, 26.7, 27.5, 31.1, 34.1, 34.6, 36.9, 37.0, 39.9, 40.3, 42.5, 42.6, 47.4, 50.2, 50.3, 55.4, 79.4 (C-24), 73.8 (C-25), 75.5 (C-20), 218.0 (C-3). ESI-MS, *m*/*z*: 442.1 $[M (476) – H_2O – OH + 1]^+$ (100%).

24,25(*R*,*S*)-24,25-Epoxy-20(*S*)-hydroxydammar-3-one (6). *a*) A solution of **1** (1 mmol, 0.44 g) in Me_2CO (10 mL) was stirred continuously, treated in small portions with a solution of dimethyldioxirane (1.1 eq.) in Me_2CO, and stirred for 3 h until the starting material disappeared completely (TLC monitoring). The solvent was evaporated *in vacuo*. The product was chromatographed over a column of Al_2O_3 with elution successively by C_6H_6 and $CHCl_3$. Yield 0.36 g (83%), mp 141–143°C. PMR spectrum (δ, ppm , J/Hz): 0.93 (3H × 0.8, s, CH_3), 0.94 (3H × 0.2, s, CH_3), 0.99 (3H × 0.8, s, CH_3), 1.01 (3H × 0.2, s, CH_3), 1.03 (3H × 0.8, s, CH_3), 1.04 (3H × 0.2, s, CH_3), 1.08 (3H × 0.8, s, CH_3), 1.09 (3H × 0.2, s, CH_3), 1.12 (3H × 0.8, s, CH_3), 1.13 (3H × 0.2, s, CH_3), 1.14 (3H × 0.8, s, CH_3), 1.15 (3H × 0.2, s, CH_3), 1.19 (3H × 0.8, s, CH_3), 1.21 (3H × 0.2, s, CH_3), 1.25–1.70 (8H, m, CH, CH_2),1.72 (3H, s, CH_3), 1.73–1.95 (14H, m, CH, CH_2), 2.39–2.53 (6H, m, CH, CH_2), 3.75 (1H × 0.8, t, J = 7.2, H-24), 3.75 (1H × 0.2, dd, J_1 = 5.5, J_2 = 9.4, H-24). ^{13}C NMR spectrum (δ, ppm): 15.1 (0.8 × C), 15.2 (0.2 × C), 16.0 (0.8 × C), 16.1 (0.2 × C), 16.3, 19.7, 21.0, 22.1 (0.8 × C), 22.3 (0.2 × C), 23.6, 24.1 (0.2 × C), 24.3 (0.8 × C), 25.7 (0.8 × C), 25.8 (0.2 × C), 26.1 (0.8 × C), 26.4 (0.2 × C), 26.7 (0.8 × C), 26.8 (0.2 × C), 27.0 (0.8 × C), 27.2 (0.2 × C), 27.4, 27.8, 31.4, 34.1, 34.6 (0.8 × C), 34.8 (0.2 × C), 35.7, 36.8, 39.9 (0.8 × C), 40.3 (0.2 × C), 43.0 (0.2 × C), 43.1 (0.8 × C), 47.4, 49.5, 49.8 (0.2 × C), 49.9 (0.8 × C), 50.0, 50.1, 55.3, 70.3, 71.4 (C-20), 83.3 (0.8 × C), 86.3 (0.2 × C), 86.4 (0.8 × C), 86.5 (0.2 × C), 217.9 (C-3).

b) A solution of **1** (1 mmol, 0.44 g) in anhydrous $CHCl_3$ (20 mL) was treated with *m*-CPBA (2.6 mmol, 0.40 g), stirred in the dark for 1 d, neutralized with KI solution (10%, 2 × 20 mL) and H_2O (2 × 30 mL), and dried over $MgSO_4$. The solvent was evaporated in vacuo. The product was chromatographed over a column of Al_2O_3 with elution successively by C_6H_6 and $CHCl_3$. Yield 0.40 g (87%), mp 142–144°C. $C_{30}H_{50}O_3$. PMR spectrum (δ, ppm , J/Hz): 0.93 (3H × 0.6, s, CH_3), 0.94 (3H × 0.4, s, CH_3), 0.99 (3H × 0.6, s, CH_3), 1.01 (3H × 0.4, s, CH_3), 1.03 (3H × 0.6, s, CH_3), 1.04 (3H × 0.4, s, CH_3), 1.08 (3H × 0.6, s, CH_3), 1.09 (3H × 0.4, s, CH_3), 1.12 (3H × 0.6, s, CH_3), 1.13 (3H × 0.4, s, CH_3), 1.14 (3H × 0.6, s, CH_3), 1.15

(3H × 0.4, s, CH_3), 1.19 (3H × 0.6, s, CH_3), 1.21 (3H × 0.4, s, CH_3), 1.25–1.70 (8H, m, CH, CH_2),1.72 (3H, s, CH_3), 1.73–1.95 (14H, m, CH, CH_2), 2.39–2.53 (6H, m, CH, CH_2), 3.75 (1H × 0.6, t, J_1 = 7.2, H-24), 3.75 (1H × 0.4, dd, J_1 = 5.5, J_2 = 9.4, H-24). ^{13}C NMR spectrum (δ, ppm): 15.1 (0.6 × C), 15.2 (0.4 × C), 16.0 (0.6 × C), 16.1 (0.4 × C), 16.4, 19.7, 21.0, 22.1 (0.6 × C), 22.3 (0.4 × C), 23.4 (0.6 × C), 23.6 (0.4 × C), 24.1 (0.4 × C), 24.3 (0.6 × C), 25.7 (0.6 × C), 25.8 (0.4 × C), 26.1 (0.6 × C), 26.4 (0.4 × C), 26.7 (0.6 × C), 26.8 (0.4 × C), 27.0 (0.6 × C), 27.2 (0.4 × C), 27.5, 27.8, 31.4, 34.1, 34.6 (0.6 × C), 34.8 (0.4 × C), 35.7, 36.8, 39.9 (0.6 × C), 40.3 (0.4 × C), 43.0 (0.4 × C), 43.1 (0.6 × C), 47.4, 49.5, 49.8 (0.4 × C), 49.9 (0.6 × C), 50.0, 50.1, 55.3, 70.3, 71.4 (C-20), 83.3 (0.6 × C), 86.3 (0.4 × C), 86.4 (0.6 × C), 86.5 (0.4 × C), 218.1 (0.6 × C-3), 218.2 (0.4 × C).

Method for Synthesizing 7 and 8. A solution of **6** (1 mmol, 0.46 g) in anhydrous CH_2Cl_2 was stirred, cooled to 0–5°C, treated in portions with $NaBH_4$ (2 mmol, 0.07 g), and stirred for 20 min at 5°C. The excess of $NaBH_4$ was neutralized with HCl solution (1 M). The organic layer was separated and dried over Na_2SO_4. The solvent was evaporated in vacuo. The product was purified by column chromatography over SiO_2 using hexane:EtOAc (5:1) eluent. Total yield of **7** and **8**, 0.42 g (92%).

20(*S*)-Hydroxydammar-3*β*,25-diol (7). Yield 0.27 g (60%), mp 123–125°C, $[\alpha]_D^{20}$ –70° (*c* 1.0, $CHCl_3$). $C_{30}H_{54}O_3$. PMR spectrum (δ, ppm , J/Hz): 0.71–0.76 (2H, m, CH), 0.77 (3H, s, CH_3), 0.84 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.96 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.14 (3H, s, CH_3), 1.22 (3H, s, CH_3), 1.25–1.37 (10H, m, CH, CH_2), 1.39–1.82 (17H, m, CH, CH_2), 2.05 (3H, br.s, OH), 3.19 (1H, dd, J_1 = 11.2, J_2 = 4.9, H-3). ^{13}C NMR spectrum (δ, ppm): 15.4, 15.5, 16.2, 16.5, 18.3, 18.5, 21.6, 24.9, 25.5, 27.4, 27.5, 28.0, 29.3, 29.4, 31.2, 35.2, 37.1, 38.9, 39.0, 40.4, 41.0, 42.3, 44.5, 49.8, 50.3, 50.6, 55.9, 71.1 (C-20), 75.5 (C-25), 78.9 (C-3). ESI-MS, *m*/*z* 445.6 [M (462) – H_2O + 1]$^+$ (~10%), 427.8 [M (462) –2H_2O + 1]$^+$ (100%).

20(*S*)-Hydroxydammar-24,25-epoxy-3*β*-ol (8). Yield 0.14 g (32%) as a 3:1 mixture of two isomers, mp 158–161°C, $[\alpha]_D^{20}$ –60° (*c* 1.0, $CHCl_3$). $C_{30}H_{52}O_3$. PMR spectrum (δ, ppm , J/Hz): 0.70–0.75 (2H, m, CH), 0.77 (3H, s, CH_3), 0.84 (3H × 0.7, s, CH_3), 0.85 (3H × 0.3, s, CH_3), 0.87 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.12 (3H × 0.7, s, CH_3), 1.13 (3H × 0.3, s, CH_3), 1.21 (3H, s, CH_3), 1.73–1.69 (9H, m, CH, CH_2), 1.69–1.91 (15H, m, CH, CH_2), 2.12 (1H, br.s, CH), 3.19 (1H, dd, J_1 = 5.2, J_3 = 10.3, H-3), 3.39 (2H, br.s, OH), 3.65 (1H × 0.3, dd, J_1 = 5.4, J_2 = 9.1, H-24), 3.73 (1H × 0.7, t, J_1 = 7.2, H-24). ^{13}C NMR spectrum (δ, ppm): 15.3, 15.4, 16.2, 16.5, 18.3, 21.5 (0.7 × C), 21.8 (0.3 × C), 23.5, 24.3, 25.7, 26.1, 27.7, 27.4, 27.3, 28.0, 31.4, 35.3, 35.7, 37.1, 38.9, 39.0, 40.3, 42.8 (0.3 × C), 42.9 (0.7 × C), 49.5, 49.8 (0.3 × C), 50.0 (0.7 × C), 50.7 (0.7 × C), 50.8 (0.3 × C), 55.8, 70.3 (0.3 × C-20), 71.43 (0.7 × C-20), 78.9 (C-3), 86.5 (0.3 × C-24), 86.4 (0.7 × C-24), 86.3 (0.3 × C-25), 83.3 (0.7 × C-25). ESI-MS, *m*/*z* 443.4 [M (460.4) – H_2O + 1]$^+$ (100%), 425.6 [M (460) –2H_2O + 1]$^+$ (100%).

20(*S*)-Hydroxydammar-24,25-epoxy-3*β*-acetate (9). A mixture of **7** (1 mmol, 0.46 g) and anhydrous Py (2 mL) was treated with dimethylaminopyridine (0.64 g) and Ac_2O (2 mL), stirred at room temperature for 2 h, and poured into H_2O (20 mL). The precipitate was filtered off, washed until neutral, and purified by column chromatography over SiO_2. Yield 0.43 g (94%) as a 3:1 mixture of two isomers, mp 110–111°C, $[\alpha]_D^{20}$ –9° (*c* 1.0, $CHCl_3$). $C_{32}H_{54}O_4$. PMR spectrum (δ, ppm , J/Hz): 0.82–0.84 (2H, m, CH), 0.85 (3H × 0.7, s, CH_3), 0.86 (3H × 0.3, s, CH_3), 0.87 (3H × 0.7, s, CH_3), 0.88 (3H × 0.3, s, CH_3), 0.95 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.11 (3H × 0.7, s, CH_3), 1.12 (3H × 0.3, s, CH_3), 1.13 (3H × 0.7, s, CH_3), 1.14 (3H × 0.3, s, CH_3), 1.18 (3H × 0.7, s, CH_3), 1.20 (3H × 0.3, s, CH_3), 2.01 (3H, s, OCO$C\underline{H}_3$), 1.24–1.38 (10H, m, CH, CH_2), 1.41–1.94 (18H, m, CH, CH_2), 3.72 (1H × 0.3, t, J = 7.2, H-24), 3.64 (1H × 0.7, dd, J_1 = 9.9, J_2 = 5.3, H-24), 4.48 (1H, m, H-3). ^{13}C NMR spectrum (δ, ppm): 15.4 (0.3 × C), 15.5 (0.7 × C), 16.0, 16.3 (0.7 × C), 16.4 (0.3 × C), 18.3, 21.3 (0.7 × C), 21.5 (0.3 × C), 21.8, 23.5, 23.7, 24.1, 24.3, 25.7, 25.8, 26.1, 26.4 (0.3 × C), 26.9 (0.7 × C), 27.3 (0.7 × C), 27.4 (0.3 × C), 28.0, 31.4, 35.3 (0.7 × C), 35.7 (0.3 × C), 37.1, 38.9, 39.0, 40.4, 42.8 (0.3 × C), 42.9 (0.7 × C), 49.6, 49.8 (0.3 × C), 50.0 (0.7 × C), 50.8 (0.3 × C), 50.7 (0.7 × C), 55.9, 70.3 (0.3 × C-20), 71.4 (0.7 × C-20), 80.9 (C-3), 86.5 (0.3 × C-24), 86.4 (0.7 × C-24), 86.3 (0.3 × C-25), 83.3 (0.7 × C-25), 170.9 (C=O). ESI-MS, *m*/*z*: 442.6 [M (502) – OH + 1]$^+$ (~10%), 425.5 [M – OH – H_2O + 1]$^+$ (100%).

Method for Synthesizing 10 and 11. A solution of **1** (1 mmol, 0.44 g) or **6** (1 mmol, 0.46 g) in Py:MeOH (1:1, 20 mL) was treated with NH_2OH·HCl (2.6 mmol, 1 g), refluxed for 6 h, and poured into HCl solution (20 mL, 5%). The precipitate was filtered off, washed with H_2O, dried in air, and purified by column chromatography over SiO_2 using $CHCl_3$ eluent.

20(*S*)-Hydroxydammar-3-oxime-24-ene (10). Yield 0.37 g (85%), mp 187–188°C, R_f 0.65, $[\alpha]_D^{20}$ +14° (*c* 1.8, $CHCl_3$). $C_{30}H_{51}NO_2$. PMR spectrum (δ, ppm , J/Hz): 0.89 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.01 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.21–1.60 (14H, m, CH, CH_2), 1.64 (3H, s, CH_3), 1.69 (3H, s, CH_3), 1.71–1.89 (11H, m, CH, CH_2), 2.01–2.12 (2H, m, CH, CH_2), 2.20–2.39 (1H, m, CH), 5.13 (1H, t, J = 7.1, H-24), 7.69 (1H, br.s, OH). ^{13}C NMR spectrum

(δ, ppm): 15.3, 15.84, 16.3, 17.2, 17.6, 19.0, 21.7, 22.5, 22.8, 24.7, 25.4, 25.7, 27.3, 27.5, 31.1, 34.8, 37.1, 39.0, 40.4, 40.5, 42.3, 42.6, 49.7, 50.2 (2C), 56.0, 75.3 (C-20), 124.7 (C-24), 131.5 (C-25), 167.0 (C-3).

20(*S*)-Hydroxydammar-24,25-epoxy-3-oxime (11). Yield 0.38 g (82%) as a 3:1 mixture of two isomers, mp 144–146°C, $[\alpha]_D^{20}$ –42° (*c* 1.0, $CHCl_3$). $C_{30}H_{51}NO_3$. PMR spectrum (δ, ppm , J/Hz): 0.86 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.96 (3H, s, CH_3), 0.98 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.13 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.16 (3H, s, CH_3), 1.19 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.26 (3H, s, CH_3), 2.42 (2H, m, H_b-2, H_b-2 isomer), 2.97 (2H, m, H_a-2, H_a-2 isomer), 3.65 (1H, dd, J_1 = 9.9, J_2 = 5.3, H-24), 3.73 (1H, t, J = 7.2, H-24). ^{13}C NMR spectrum (δ, ppm): 15.8 (0.7 × C), 15.9 (0.3 × C), 16.3 (0.7 × C), 16.4 (0.3 × C), 17.4, 19.1, 21.9 (0.7 × C-24), 22.1 (0.3 × C-24), 22.9, 23.5, 24.1, 24.3, 25.7 (0.7 × C-24), 25.8 (0.3 × C-24), 26.2, 26.4, 27.0 (0.7 × C-24), 27.1 (0.3 × C-24), 27.4 (0.7 × C), 27.5 (0.3 × C), 27.5, 27.8, 29.7, 31.4, 34.9, 35.8, 37.2, 39.0, 40.4 (0.7 × C), 40.5 (0.3 × C), 42.9 (0.7 × C-24), 43.0 (0.3 × C-24), 49.6 (0.7 × C-24), 49.8 (0.3 × C-24), 50.1, 50.4 (0.7 × C-24), 50.5 (0.3 × C-24), 56.0, 70.3 (0.3 × C-20), 71.5 (0.7 × C-20), 83.4 (0.7 × C-24), 86.4 (0.3 × C-24), 86.5 (0.7 × C-25), 86.6 (0.3 × C-25), 168.0 (C-3). ESI-MS, *m*/*z* 474.3 $[M + 1]^+$ (100%), 457.2 $[M – H_2O + 1]^+$ (~5%), 439.3 $[M – 2H_2O + 1]^+$ (~10%).

2,3-Indolo-20(*S*)-hydroxydammar-24-ene (12). Compound **1** (1 mmol, 0.43 g) in HOAc (20 mL) was treated with phenylhydrazine (3.5 mmol, 0.35 mL), refluxed for 15 h, and poured into stirring cold H_2O. The precipitate was filtered off, washed with H_2O, dried in air, and purified by column chromatography over Al_2O_3 using C_6H_6 eluent. Yield 0.36 g (86%), mp 86–88°C, $[\alpha]_D^{20}$ +76° (*c* 0.6, $CHCl_3$). $C_{36}H_{53}NO$. PMR spectrum (δ, ppm , J/Hz): 0.90 (3H, s, CH_3), 0.91 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.19 (3H, s, CH_3), 1.29 (3H, s, CH_3), 1.32–1.60 (14H, m, CH, CH_2), 1.65 (3H, s, CH_3), 1.71 (3H, s, CH_3), 1.75–2.81 (12H, m, CH, CH_2), 5.15 (1H, t, J = 7.1, H-24), 7.01–7.73 (4H, m, $H_{arom.}$), 8.02 (1H, br.s, NH). ^{13}C NMR spectrum (δ, ppm): 15.3, 15.9, 16.4, 17.7, 19.3, 22.0, 23.2, 25.7, 27.0, 27.4, 27.7, 30.9, 31.7, 33.7, 34.2, 34.8, 37.6, 38.3, 40.5, 40.9, 44.5, 49.3, 49.8, 50.2, 53.8, 76.6 (C-20), 107.1 ($C_{arom.}$), 110.3 (C-2), 118.0 ($C_{arom.}$), 121.0 ($C_{arom.}$), 123.9 (C-24), 127.3 ($C_{arom.}$), 130.0 (C-25), 136.2 ($C_{arom.}$), 137.3 ($C_{arom.}$), 140.9 (C-3).

The method for testing the *in vitro* antitumor activity of **1**–**4**, **6**, **7**, and **12** on 60 cell lines of nine human tumors is available at the website www.dtp.nci.nih.gov.

The cytotoxicity of **1**, **5**–**9**, and **11** was studied at the IC VAST using epidermoid cancer KB cells and the standard MTT test [19] that is based on the ability of dehydrogenases of living cells to transform the colorless form of MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] into blue crystals of formazan that are soluble in DMSO.

Cell lines of epidermal cancer KB were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were grown in DMEM (Dulbecco's modified Eagle's medium) with added fetal calf serum (10%) and penicillin and streptomycin (1%) at 37°C, 5% atmospheric CO_2, and 95% humidity. Cells were treated with the studied compounds at the required concentration after 24 h and incubated for 48 h. MTT reagent (5 mg/mL) was added at the end of the incubation period. Optical absorption was measured on a Tecan Geniou instrument at 450 nm. Results were evaluated against control samples (without added compound) and expressed as the cell survival index in percent that was calculated using the formula At/Ac × 100, where At is the optical absorption of the test sample and Ac, that of the control. The IC_{50} value was determined from a dose–effect curve as the lowest concentration at which growth of the treated cells was reduced by 50% compared with the control cells. A compound was considered active if $IC_{50} < 4$ μg/mL [16].

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REFERENCES

1. T. Akihisa, H. Tokuda, M. Ukiya, T. Suzuki, F. Enjo, K. Koike, T. Nikado, and H. Nishino, *Chem. Pharm. Bull.*, **52**, 153 (2004).
2. F. Inada, M. Somekawa, and H. Murata, *Chem. Pharm. Bull.*, **41**, 617 (1993).

3. G. V. Platonov, A. D. Zorina, M. A. Gordon, N. P. Chizhov, L. V. Balykina, Yu. D. Mikhailov, D. R. Ivanen, T. K. Qui, and A. G. Shavva, *Khim.-farm. Zh.*, **29**, 42 (1995).
4. T. Akihisa, J. Ogihara, J. Kato, K. Yasukawa, M. Ukiya, S. Yamanouchi, and K. Oishi, *Lipids*, **36**, 507 (2001).
5. D. Scholz, K. Baumann, M. Grassberger, B. Wolff-Winiski, G. Rihs, H. Walter, and J. G. Meingassner, *Bioorg. Med. Chem. Lett.*, **14**, 2983 (2004).
6. J.-M. Zhao, N. Li, C.-F. Wu, H.-R. Piao, and Y.-Q. Zhao, *Bioorg. Med. Chem. Lett.*, **21**, 1027 (2011).
7. N. H. Tung, G. Y. Song, J.-A. Kim, J.-H. Hyun, H.-K. Kang, and Y. H. Kim, *Bioorg. Med. Chem. Lett.*, **20**, 309 (2010).
8. J. Phongmaykin, T. Kumamoto, T. Ishikawa, E. Saifah, and R. Suttisri, *Nat. Prod. Res.*, **25** (17), 1621 (2011).
9. M. Ukiya, T. Kikuchi, H. Tokuda, K. Tabata, Y. Kimura, T. Arai, Y. Ezaki, O. Oseto, T. Suzuki, and T. Akihisa, *Chem. Biodiversity*, **7**, 1871 (2010).
10. D. Schmitz, J. Zapp, and R. Bernhardt, *FEBS J.*, **279**, 1663 (2012).
11. H. Hasegawa, *J. Pharmacol. Sci.*, **95**, 153 (2004).
12. M. C. Alley, D. A. Scudiero, P. A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, and M. R. Boyd, *Cancer Res.*, **48** (3), 589 (1988).
13. M. R. Grever, S. A. Schepartz, and B. A. Chabner, *Semin. Oncol.*, **19**, 622 (1992).
14. M. R. Boyd and K. D. Paull, *Drug. Dev.*, **34**, 91 (1995).
15. R. H. Shoemaker, *Nat. Rev. Cancer*, **6**, 813 (2006).
16. C. C. Lee and P. Houghton, *J. Ethnopharmacol.*, **100**, 237 (2005).
17. L. Q. Tran and K. Q. Tran, *J. Chem.*, **36**, 8 (1998).
18. A. D. Zorina, L. V. Balykina, O. V. Nazarova, and A. A. Rebezov, *Zh. Prikl. Khim.*, **79**, 663 (2006).
19. D. Sladowski, S. J. Steer, R. H. Chothier, and M. Balls, *J. Immunol. Methods*, **157**, 203 (1993).