Plant-Derived Triterpenoids as Potential Antineoplastic Agents

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Abstract: Man has relied on plants as a source of medicinal agents for centuries. Today, with the specter of antibiotic resistance, emerging infectious diseases, and cancers, phytochemicals continue to provide new structural leads for the chemotherapeutic industry. A number of triterpenoids have shown promise as antineoplastic agents. Members of the cycloartane, lupane, ursane, oleanane, friedelane (especially quinone methides), dammarane, cucurbitacin, and limonoid triterpenoids, have demonstrated anti-proliferative activity on various cancer cell lines. This review covers the recent developments regarding antineoplastic/cytotoxic triterpenoids, excluding saponins, from higher plants.

Keywords: Triterpenoids, cytotoxic, antineoplastic, cancer.

INTRODUCTION

For centuries man has relied on natural products as sources for obtaining medicinal agents, most of which were obtained from higher plants [1]. Even today, about 80% of the world's population relies predominantly on plants and plant extracts for health care [1]. In "developed countries" plants are important sources of medicines. Thus, for example, there are more than 120 important prescription medicines in the United States that are plant based, which represents about 25% of the total drugs in use. Not only do higher plants continue to serve as important sources of new drugs, but phytochemicals derived from them are also extremely useful as lead structures for synthetic modification and optimization of bioactivity [1].

In terms of cancer treatment, there are serious limitations in chemotherapy, namely the lack of selectivity of active ingredients and the development of resistance by cancer cells to these chemicals [2]. Current chemotherapeutic agents destroy both cancerous and non-cancerous cells. Thus, there is an urgent need to find new chemical agents that can differentiate between normal cells and cancer cells in order to selectively kill the cancerous cells with reduced toxicity.

The potential interest of plant-derived triterpenoids as antineoplastic agents is reflected in the number of scientific papers appearing in the field, and this has expanded rapidly. An analysis of publications on antineoplastic triterpenoids abstracted in PubMed over the past 15 years reveals an exponential rate of increase in the number of articles in this area of research [see Fig (**1**)].

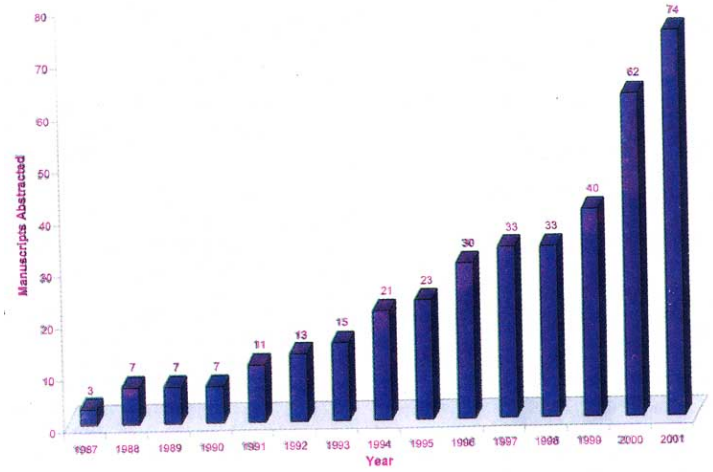

Fig. (1). Antineoplastic triterpenoids manuscripts abstracted in PubMed for the years 1987-2001.

This review covers cytotoxic or antineoplastic triterpenoids of plant origin excluding saponins and triterpenoids of animal and fungal origins. In addition, neither chemopreventive nor antitumorigenic activity is covered. The structural features and proposed mechanism of action of the following classes of triterpenoid are examined: Cycloartanes, lupanes, ursanes, oleananes, friedelanes, dammaranes, cucurbitacins, and limonoids. Cytotoxicity data for the triterpenoids have been summarized in Table 1 along with Paclitaxel (Taxol®) for comparison.

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Table 1. Cytotoxic Activities of Triterpenoids

Compound	Cytotoxicity (IC_{50}, μM)	Cell Line	Ref
Cycloartane Triterpenoids			
1	9.54	26-L5	[3]
2	5.42	26-L5	[3]
3	17.00	Erlich ascites	[4]
4	7.50	Erlich ascites	[4]
5	163	CCL-81	[5]
6	90.1	CCL-81	[5]
7	1.1	KB	[6]
	1.4	A549	[6]
	1.6	HTC-8	[6]
	1.7	P-388	[6]
Lupane Triterpenoids			
8	39.4	MEL-2	[8]
11	0.821	Co-115	[12]
	2.6	MEL-2	[8]
	20	LN-229	[17]
	25	U87MG	[17]
	25	T98G	[17]
	70	LN-18	[17]
	100	LN-308	[17]
	34	Hep G2	[14]
	9.61	MDA-MB-231	[14]
	4	A549	[13]
	8.3	SK-OV-3	[13]
	5.5	SK-MEL-3	[13]
	8.3	XF498	[13]
	8.1	HCT-15	[13]
12	10.2	P-388	[23]
	13	LL/2	[23]
	4.0	human large-cell lung carcinoma	[23]
13	8.0	U373	[24]
Ursane Triterpenoids			
16	14	UMR106	[26]
17	30	NUGC-3	[37]
	7	Hep G2	[28]
	4.05	A549	[28]
	3.3	HeLa	[28]
	27.4	K562	[28]
	7.2	HTC-15	[29]
	7.0	UISO	[29]

(Table 1). contd.....

Compound	Cytotoxicity (IC_{50}, μM)	Cell Line	Ref
Ursane Triterpenoids			
17	7.0	OVCAR-5	[29]
	7.7	B16	[92]
	0.85	HL-60	[27]
	7.9	HL-60	[39]
	9.2	A549	[13]
	7.9	SK-OV-3	[13]
	10.1	SK-MEL-3	[13]
	9.9	XF498	[13]
	9.6	HTC-15	[13]
20	10.2	Hep G2	[28]
	10.6	A549	[28]
	2.1	HeLa	[28]
	9.1	K562	[28]
	9.3	A549	[13]
	8.3	SK-OV-3	[13]
	10.8	SK-MEL-3	[13]
	11.6	XF498	[13]
	9.9	HTC-15	[13]
21	17.6	ME-180	[33]
	25.0	DU145	[33]
	29.0	MCF7	[33]
	14.6	M-14	[33]
22	37.6	SK-OV-3	[13]
24	31	HL-60	[36]
	32	CCRF-CEM	[36]
27	1.43	Hep G2	[43]
	15.3	MDA-MB-231	[43]
cis-**28**	1.5	P-388	[44]
trans-**28**	3.2	P-388	[44]
cis-**29**	25	KB	[45]
trans-**29**	12	KB	[45]
cis-**30**	4.2	A549	[46]
	3.7	HTC-15	[46]
	0.9	MCF7	[46]
	6.6	HT-1197	[46]
trans-**30**	9.7	A549	[46]
	2.8	HTC-15	[46]
	8.8	MCF7	[46]
	8.6	HT-1197	[46]
cis-**31**	7.3	A549	[46]

(Table 1). contd.....

Compound	Cytotoxicity (IC_{50}, μM)	Cell Line	Ref
Ursane Triterpenoids			
cis-**31**	4.2	HTC-15	[46]
	1.4	MCF7	[46]
	7.0	HT-1197	[46]
trans-**31**	3.7	A549	[46]
	4.4	HTC-15	[46]
	0.9	MCF7	[46]
	5.0	HT-1197	[46]
Oleanane Triterpenoids			
32	35.9	A549	[13]
	27.2	SK-OV-3	[13]
	40.5	SK-MEL-3	[13]
	26.5	HCT-15	[13]
33	35.7	KB	[49]
	74.1	P-388	[49]
34	7.9	HCT-15	[51]
	8.5	UISO	[51]
	11.6	KB	[51]
35	41.0	A549	[13]
	38.9	SK-OV-3	[13]
	41.9	SK-MEL-3	[13]
	32.4	HCT-15	[13]
40	6.6	MDA-MB-231	[53]
41	1.6	MDA-MB-231	[53]
42	2.6	A549	[13]
	2.6	SK-OV-3	[13]
	2.8	SK-MEL-3	[13]
	2.9	XF498	[13]
	2.8	HCT-15	[13]
44	4.91	KB	[54]
45	3.74	KB	[54]
cis-**46**	2.7	A549	[46]
	2.1	HTC-15	[46]
	3.9	MCF7	[46]
	3.4	HT-1197	[46]
trans-**46**	1.0	A549	[46]
	2.1	HTC-15	[46]
	3.0	MCF7	[46]
	5.2	HT-1197	[46]
cis-**47**	3.0	A549	[46]
	1.9	HTC-15	[46]

(Table 1). contd.....

Compound	Cytotoxicity (IC_{50}, μM)	Cell Line	Ref
Oleanane Triterpenoids			
cis-**47**	1.9	MCF7	[46]
	1.6	HT-1197	[46]
trans-**47**	6.1	A549	[46]
	0.8	HTC-15	[46]
	1.8	MCF7	[46]
	3.4	HT-1197	[46]
48	2.9	MCF7	[55]
	3.5	PC-3	[55]
	2.1	HCT-15	[55]
49	3.5	MCF7	[55]
	2.6	PC-3	[55]
	1.3	HCT-15	[55]
Friedelane Triterpenoids			
50	0.46	A549	[56]
51	2.59	A549	[56]
52	1.40	A549	[56]
53	4.9	HEPA-2B	[57]
	8.1	KB	[57]
54	0.67	KB	[68]
	0.098	P-388	[68]
	1.7	A431	[59]
	0.5	LNCaP	[59]
	0.5	U373	[59]
	0.33	L-1210	[68]
	1.4	HeLa	[93]
	1.70	SK-MEL-28	[60]
	1.91	Hep G2	[60]
	1.49	MDA-MB-231	[62]
55	0.78	L-1210	[68]
	0.26	P-388	[68]
	1.19	KB	[68]
56	2.7	KB	[59]
	7.8	P-388	[59]
	1.3	A431	[59]
	0.7	LNCaP	[59]
	0.9	U373	[59]
	2.9	HT-29	[64]
57	1.5	HeLa	[93]
59	0.27	P-388	[68]
60	3.9	KB	[59]

(Table 1). contd.....

Compound	Cytotoxicity (IC_{50}, μM)	Cell Line	Ref
Friedelane Triterpenoids			
60	4.6	P-388	[59]
	2.5	A431	[59]
	2.3	LNCaP	[59]
	1.8	U373	[59]
61	2.0	HeLa	[67]
62	0.2	HeLa	[67]
63	2.5	HeLa	[63]
	0.6	Hep-2	[63]
64	5.31	HT-29	[64]
65	5.7	P-388	[65]
	11.5	A549	[65]
	11.5	HT-29	[65]
	11.5	MEL-28	[65]
66	11.4	P-388	[65]
	11.4	A549	[65]
	11.4	HT-29	[65]
	11.4	MEL-28	[65]
67	1.95	Hep G2	[62]
	1.23	MDA-MB-231	[62]
68	11.7	HeLa	[66]
	13.4	Hep-2	[66]
	17.2	Vero	[66]
69	6.0	P-388	[68]
	13.8	L-1210	[68]
	69	KB	[68]
70	11.4	P-388	[65]
	11.4	A549	[65]
	22.8	HT-29	[65]
	22.8	MEL-28	[65]
71	24.4	KB	[68]
72	8.9	P-388	[68]
73	3.1	P-388	[68]
	5.8	L-1210	[68]
	5.8	KB	[68]
74	20.8	KB	[69]
78	3700	P-388	[71]
Dammarane Triterpenoids			
79	9.2	P-388	[73]
80	4.6	P-388	[73]
81	10.0	P-388	[73]

(Table 1). contd.....

Compound	Cytotoxicity (IC_{50}, μM)	Cell Line	Ref
Dammarane Triterpenoids			
82	7.2	P-388	[73]
83	1.8	P-388	[74]
	1.2	KB	[74]
84	6.5	P-388	[73]
Cucurbitacins			
85	0.014	KB	[75]
86	0.0071	LNCaP	[77]
	0.0098	DU145	[77]
	0.0513	PC-3	[77]
Limonoids			
87	2.5	P-388	[81]
88	35.24	A549	[82]
	23.34	MCF7	[82]
	0.76	PC-3	[82]
	29.11	HT-29	[82]
89	2.42	A549	[82]
	4.74	MCF7	[82]
	0.574	PC-3	[82]
	3.10	HT-29	[82]
90	14.55	A549	[83]
	6.1	MCF7	[83]
	0.94	HT-29	[83]
91	6.42	A549	[82]
	4.72	MCF7	[82]
	5.42	PC-3	[82]
	7.28	HT-29	[82]
92	44.18	A549	[82]
	48.50	MCF7	[82]
	1.78	PC-3	[82]
	48.90	HT-29	[82]
93	2.2	P-388	[84]
94	0.045	P-388	[85]
	6.65	KB	[86]
95	0.02	P-388	[81]
96	0.004	P-388	[81]
	0.17	P-388	[85]
97	0.053	P-388	[85]
98	0.16	KB	86]
Miscellaneous Triterpenoids			
99	33	B16	[87]

(Table 1). contd.....

Compound	Cytotoxicity (IC_{50}, μM)	Cell Line	Ref
Miscellaneous Triterpenoids			
100	1.98	UO-31	[88]
	1.63	CCRF-CEM	[88]
	1.76	A549	[88]
	2.01	HCT-116	[88]
	2.02	U251	[88]
	1.79	OVCAR-3	[88]
101	35.5	Hep G2	[89]
	29.7	A431	[89]
102	11.7	Hep G2	[89]
	38.2	A431	[89]
104	7.83	A2780	[91]
	0.2	K562	[91]
105	1.45	A2780	[91]
	0.77	K562	[91]
106	1.18	A2780	[91]
	0.72	K562	[91]
107	1.61	A2780	[91]
	0.70	K562	[91]
108	4.81	A2780	[91]
	0.27	K562	[91]
109	0.32	A2780	[91]
	0.17	K562	[91]
Taxol®	0.0017	MCF7	[94]
	0.0036	A549	[94]
	0.0032	HT-29	[94]
	0.0035	HeLa	[95]
	0.0050	LNCaP	[95]
	20.1	MDA-MB-231	[96]
	58.7	Hep G2	[96]
	12	L-1210	[97]

*Cell lines described in this review

26-L5: murine colon carcinoma
A2780: human ovarian carcinoma
A431: human epidermoid carcinoma
A549: human lung epithelial carcinoma
B16: murine melanoma
CCRF-CEM: human lymphoblastic leukemia
Co-115: human colon carcinoma
DU 145: human prostatic metastatic carcinoma
Erlich ascites : murine mammary tumor
HCT-116: human colonic carcinoma
HCT-15: human colorectal adenocarcinoma
HeLa: human cervical adenocarcinoma
Hep G2: human hepatocellular carcinoma
Hep-2: human carcinoma of the larynx
HEPA-2B: human hepatoma
HL-60: human promyelocytic leukemia
HT-1197: human urinary bladder carcinoma
HT-29: human colorectal adenocarcinoma
HTC-15: human colon carcinoma

(Table 1). contd.....

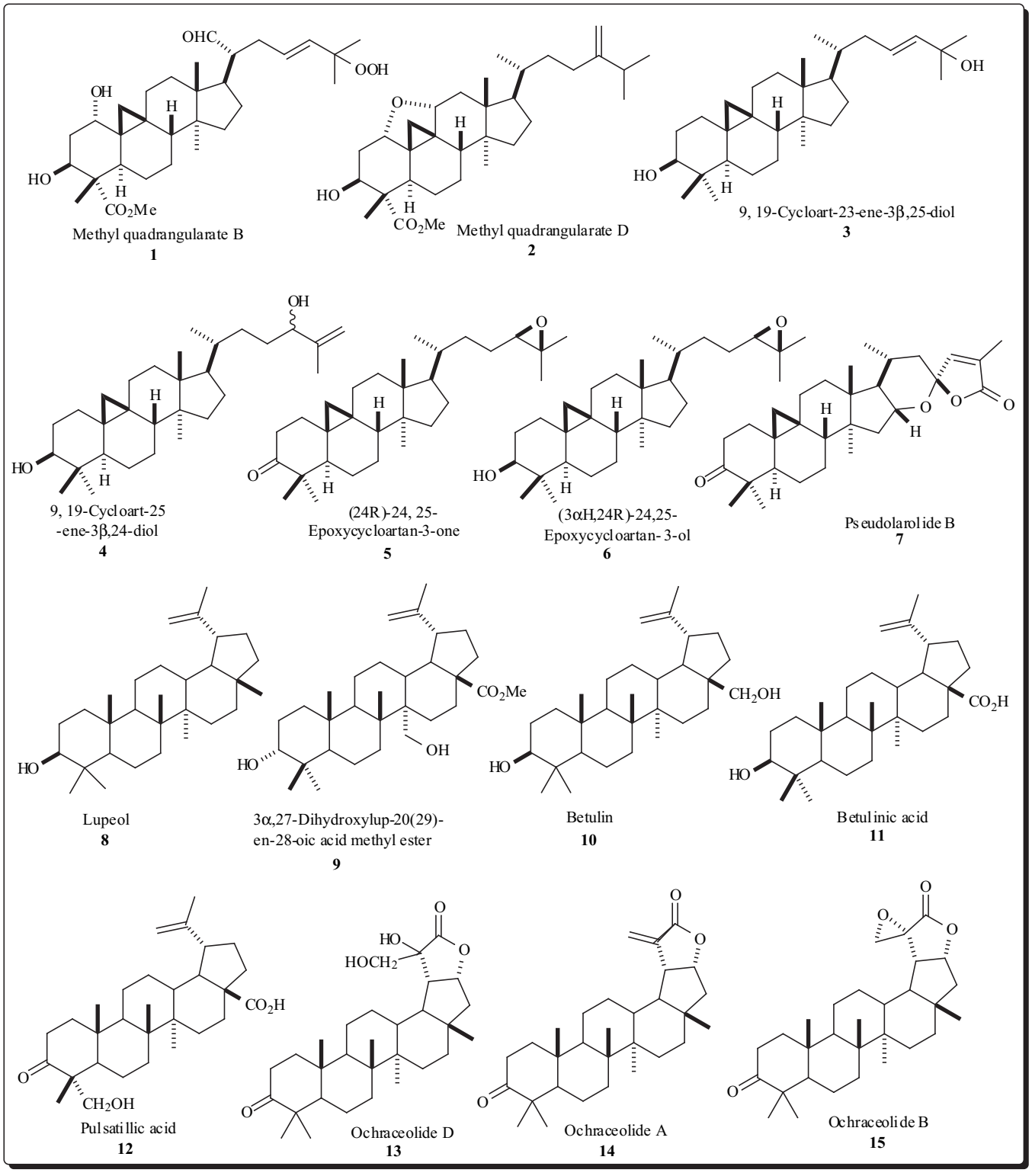
HTC-8: human colon carcinoma
K-562: human chronic myelogenous leukemia
KB : a HeLa contaminant; originally thought to be derived from an : epidermal carcinoma of the mouth
L-1210: murine lymphocytic leukemia
LL/2: Lewis lung carcinoma (murine)
LN-18: human glioblastoma
LN-229: human glioblastoma
LN-308: human malignant glioma
LNCaP: human prostatic carcinoma
M-14: human malignant melanoma
MCF7: human mammary adenocarcinoma
MDA-MB-231: human mammary adenocarcinoma
ME-180: human epidermoid cervical carcinoma
MEL-2: human melanoma
MEL-28: human melanoma
NUGC-3: human gastric adenocarcinoma
OVCAR-3: human ovarian adenocarcinoma
OVCAR-5: human ovarian carcinoma
P-388: murine lymphocytic leukemia
PC-3: human prostatic adenocarcinoma
SK-MEL-28: human malignant melanoma
SK-MEL-3: human malignant melanoma
SK-OV-3: human ovarian adenocarcinoma
T98G: human glioblastoma multiforme
U251: human glioblastoma
U373: human glioblastoma
U87MG: human glioblastoma
UISO: human squamous cervical carcinoma
UMR106: rat osteosarcoma
UO-31: renal carcinoma
Vero (CCL-81): normal monkey kidney
XF498: human CNS cancer

CYCLOARTANE TRITERPENOIDS

Methyl quadrangularate B (**1**) and methyl quadrangularate D (**2**) exhibited notable *in-vitro* cytotoxicity against murine colon carcinoma cells with IC_{50} values of 9.54 and 5.42 μM, respectively [3]. The C-25 hydroperoxide of **1** is apparently an important structural feature; the corresponding C-25 alcohol is much less toxic. A free C-3 hydroxy group in **2** gives a more cytotoxic compound than the corresponding acetate ester. Cycloartenediol **3** was shown to be less cytotoxic than the structural isomer **4** against Ehrlich ascites tumor cells [4]. The C-24,C-25 epoxide moiety increases the cytotoxicity of **5** and **6** compared to related compounds lacking the epoxide, while the C-3 hydroxyl group in **6** increases the cytotoxicity compared to the corresponding ketone **5** [5]. Cycloartane lactone **7** seems to be broadly cytotoxic (see Table 1) [6]. Thus, further supporting the view that the α,β-unsaturated lactone could be responsible for conferring activity; the saturated analog is inactive [6].

LUPANE TRITERPENOIDS

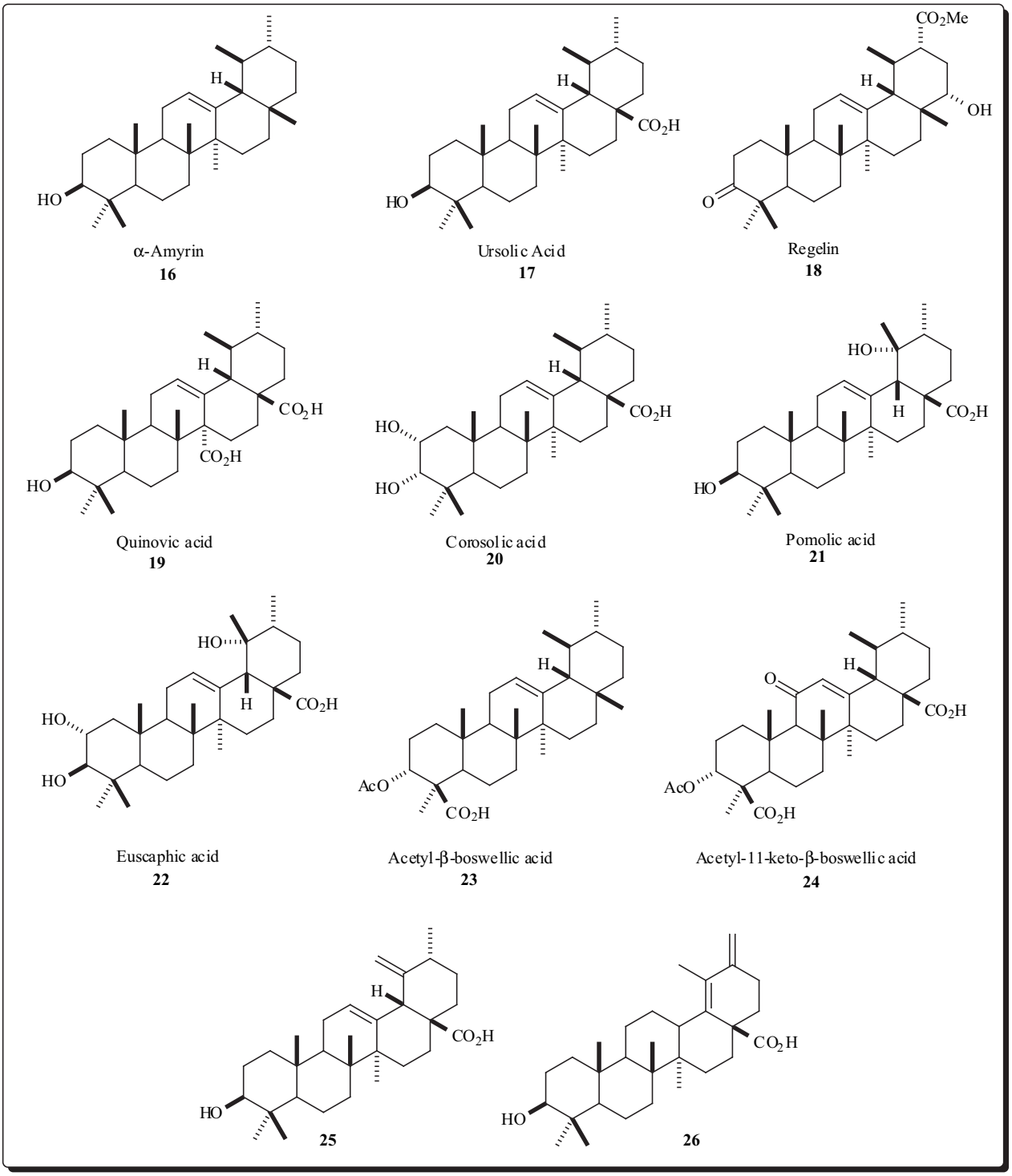
Lupeol (**8**) has exhibited *in-vivo* antitumor activity against Walker 256 carcinoma in rats [7], along with weak *in-vitro* cytotoxic activity against MEL-2 melanoma [8], and Hep G2 liver tumor [9] cells. Lupeol (**8**) [9] and related triterpenoid **9** [10] are both inhibitors of topoisomerase II. Lupeol has also been shown to inhibit farnesyl protein transferase [11]. Betulin (**10**) also was active in the Walker 256 tumor system [7], but is relatively non-cytotoxic [8]. Betulinic acid (**11**), on the other hand, has exhibited stronger cytotoxic activity (see Table 1) [8,12-14]; the activity apparently due to the presence of the carboxylic acid moiety. Betulinic acid has induced apoptosis in human melanoma

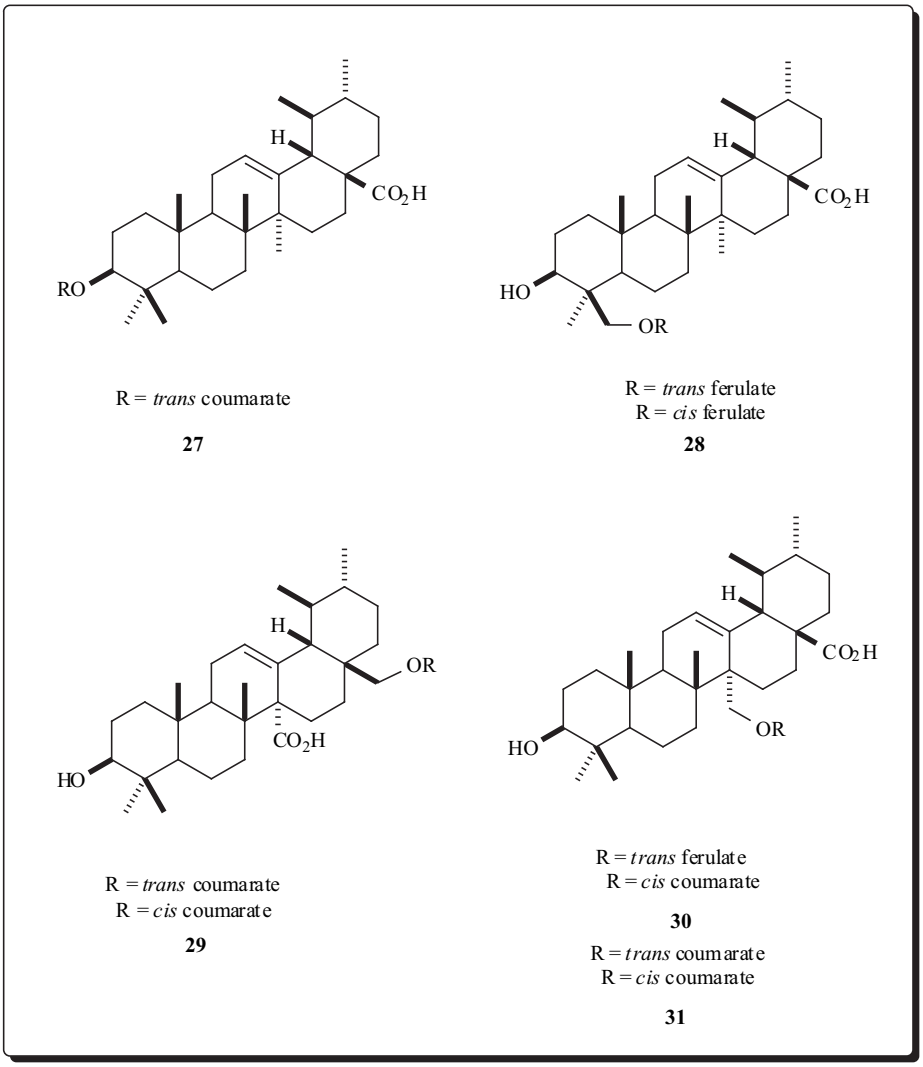


cells [15], neuroblastoma cells [16], and glioma cells [17], via a direct action on mitochondrial processes [18], in addition it is known to inhibit DNA polymerase β [19], topoisomerases I and IIα [20]. The induction of apoptosis in melanoma cells by betulinic acid has been attributed to an increase in the expression of p53 [21] Increased p53, a regulatory protein, halts the cell cycle so that cells cannot enter the S phase, thus blocking proliferation; cells either delay in the G1 phase or die by apoptosis. In addition, betulinic acid serves to inhibit aminopeptidase N activity, thereby inhibiting melanoma invasion into basement membranes, and possibly inducing apoptosis [22]. Pulsatillic acid (23-hydroxybetulonic acid, **12**) has also exhibited cytotoxic activity in a number of human tumor cell lines [23]. Ochraceolide D (**13**) exhibited cytotoxic activity against human glioblastoma cells [24], while ochraceolides A and B (**14** and **15**, respectively) were potent inhibitors of farnesyl protein transferase [23].

URSANE TRITERPENOIDS

α-Amyrin (**16**) has been reported to exhibit cytotoxic activity against Walker carcinoma 256 [25] as well as UMR106 rat osteosarcoma cells [26]. As was the case with the lupane triterpenoids, the corresponding carboxylic acid derivatives of α-amyrin showed enhanced cytotoxic activity. Thus, for example, ursolic acid (**17**) has shown good *in-vitro* cytotoxic activity against a number of human tumor cell lines [13,27-30] (e.g., IC_{50} = 3.3 and 7 μ*M* against HeLa and Hep G2 cells, respectively [28]). Similarly, regelin (**18**) [31], quinovic acid (**19**) [32], corosolic acid (**20**) [13,28], pomolic acid (**21**) [33], euscaphic acid (**22**) [13], acetyl-β-boswellic acid (**23**) [34,35], and acetyl-11-keto-β-boswellic acid (**24**) [34,36] have all exhibited cytotoxic activity against various cell lines. Furthermore, ursolic acid (**17**) has been shown to inhibit the enzymes topoisomerase I and II [20,37], DNA polymerase [37], lipoxygenase [27], and tyrosine kinase [38], and induces apoptosis [39,40] possibly involving activation of caspases [13,41]. Boswellic acid derivatives **23** and **24** have been reported to be inhibitors of topoisomerase I and IIα [20], and **24** is an inducer of



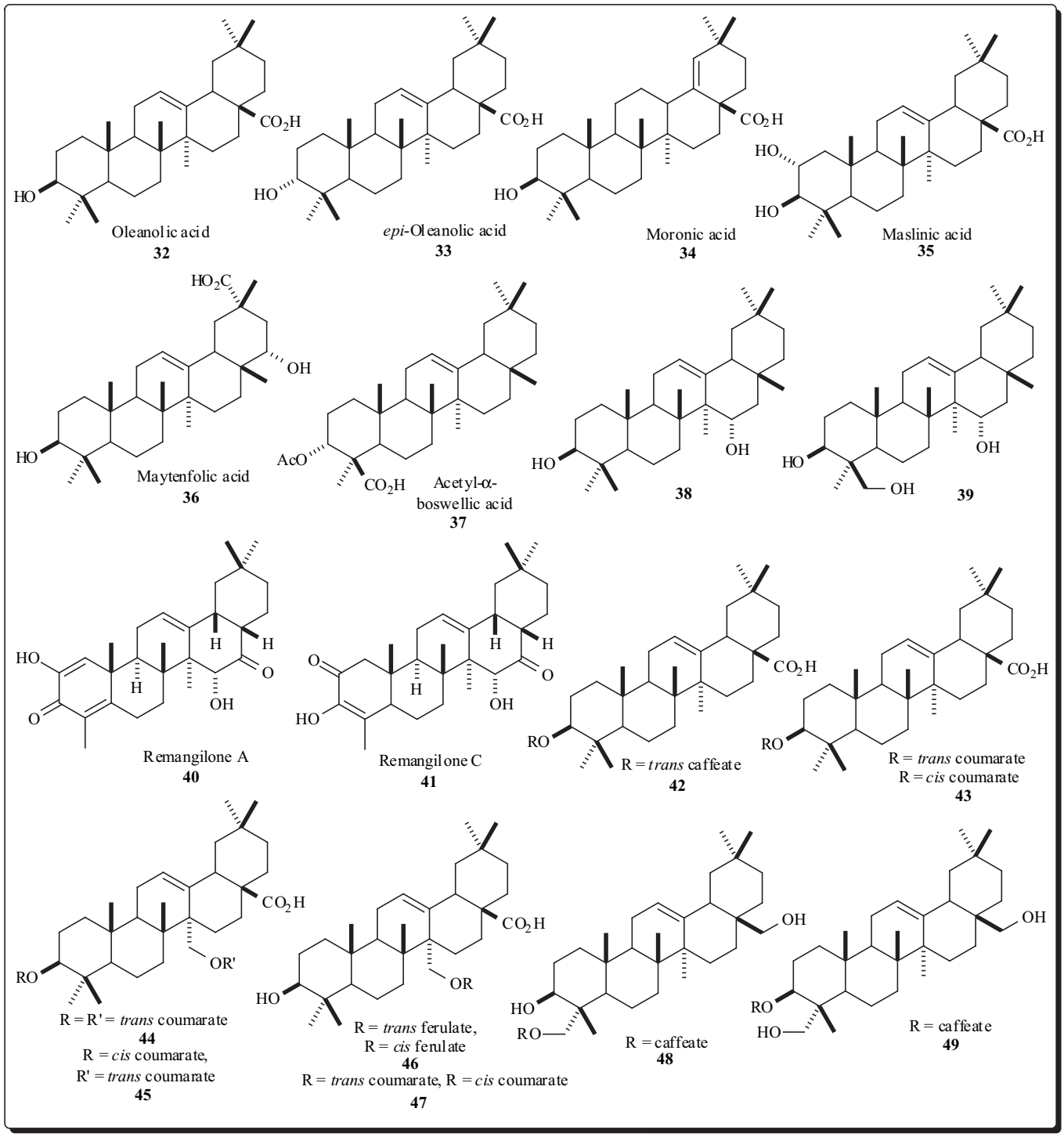


apoptosis [36]. Although not reported to be cytotoxic, ursolic acid derivatives **25** and **26** were inhibitors of DNA polymerase β [42].

The cytotoxic activity of the *p*-hydroxycinnamyl esters of ursane triterpenoids have been reported, for example ursolic acid 3-*p*-*E*-coumarate (**27**) was more cytotoxic against Hep G2 cells than ursolic acid [43]. Both *cis*- and *trans*-ferulate esters **28** were cytotoxic to P-388 cells [44] while *cis*- and *trans*-coumarate esters **29** were effective against KB cells [45]. Similarly, ferulates **30** and coumarates **31** were cytotoxic to a panel of human tumor cell lines (see Table 1) [46]. Rios and co-workers [29] have reported that coumarates **31** were not cytotoxic on the HCT-15, UISO, and OVCAR-5 cell lines. Fatty acid esterification of ursane triterpenoids appears to increase cytotoxic activity. Thus, α-amyrin-3-palmitate [26] and ursolic acid-3-palmitate [47] were both more cytotoxic than α-amyrin (**16**) and ursolic acid (**17**), respectively.

OLEANANE TRITERPENOIDS

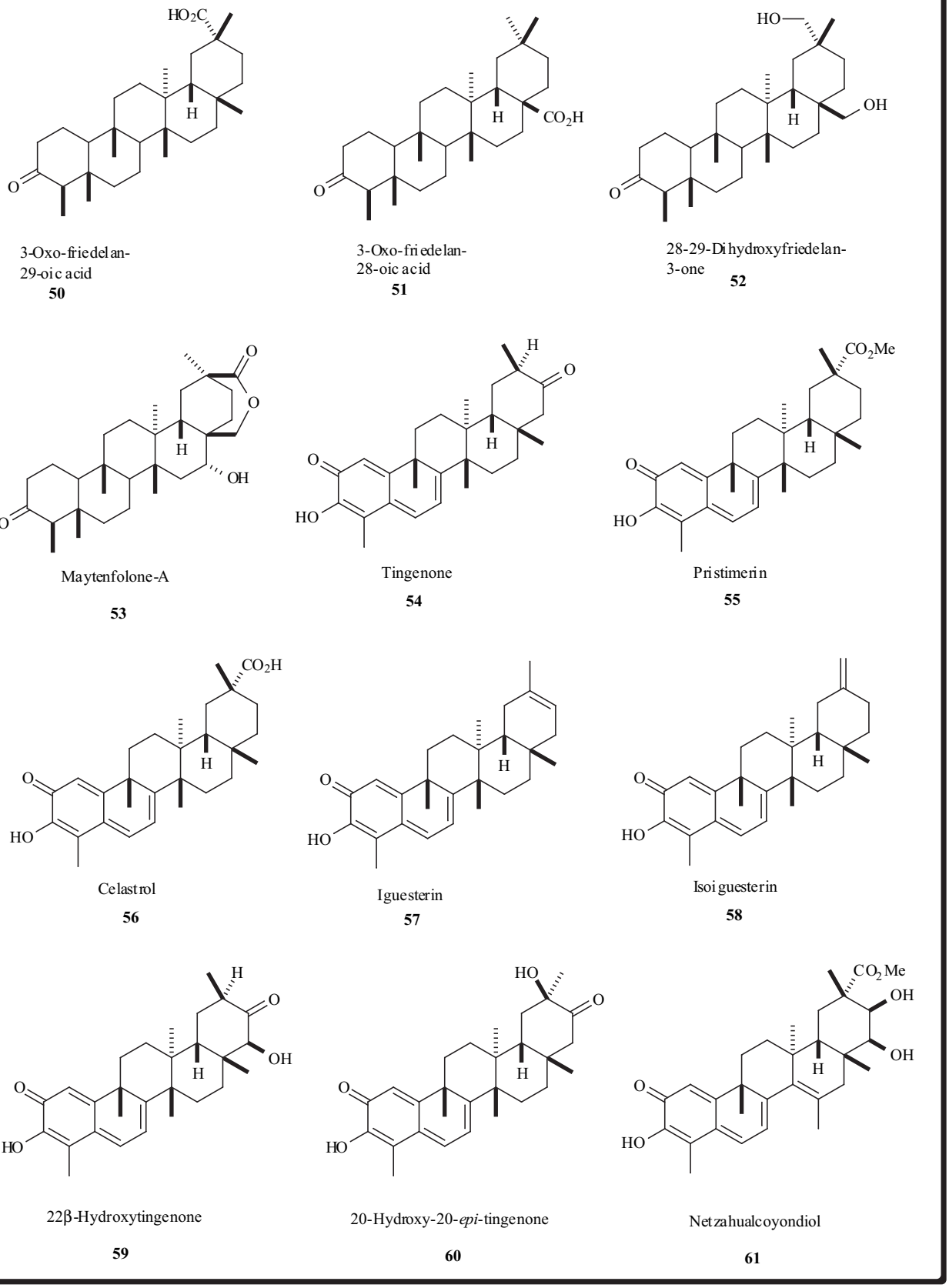
Oleanolic acid (**32**) has been reported to exhibit cytotoxic activity against a number of tumor cell lines (see Table 1) [13]. The mechanism of action of this compound appears to be inhibition of DNA polymerase [42], topoisomerase I, and topoisomerase II [20]. In addition, oleanolic acid has been shown to stimulate nitric oxide and tumor necrosis factor-α release and is able to upregulate iNOS and TNFα expression through nuclear factor-κB transactivation [48]. The 3α stereoisomer, *epi*-oleanolic acid (**33**) [49] and the Δ-18 structural isomer, moronic acid (**34**) [50] also exhibited cytotoxic activity. The *in-vitro* cytotoxic activity of maslinic acid (**35**) was similar to oleanolic acid [13], while maytenfolic acid (**36**) showed significant *in-vivo* activity against P-388 lymphocytic leukemia in mice [51]. Although cytotoxicity data for acetyl-α-boswellic acid (**37**) have not been reported, the compound has been shown to be a potent inhibitor of both topoisomerase I and topoisomerase II [20]. The minimum inhibitory concentration, MIC, of **37** necessary to prevent relaxation of supercoiled DNA by either topoisomerase I or topoisomerase II was found to be 10 μ*M* and 3 μ*M*, respectively [20]. Hydroxyamyrins **38** and **39** have also been shown to inhibit topoisomerase II [52]. Remangilones A (**40**) and C (**41**), α,β-unsaturated 24,28-dinorolean-3-ones, have demonstrated *in-vitro* cytotoxic activity toward human breast tumor cells, but reduced toxicity toward normal breast cells at equivalent concentrations [53].



As was the case for the ursane triterpenoids, *p*-hydroxycinnamyl esters of oleanolic acid and other oleanane triterpenoids exhibited potential antineoplastic activity. Oleanolic acid 3-*trans*-caffeate (**42**) is an order of magnitude more cytotoxic than oleanolic acid itself (IC_{50} = 2.6-2.9 μ*M* for **42** vs. 27-40 μ*M* for **32**) [13]. Both *cis*- and *trans*-oleanolic acid 3-*p*-coumarate (**43**) have been shown to inhibit DNA polymerase [19]. The *bis* coumarates **44** and **45** demonstrated *in-vitro* cytotoxic activity on KB cells [54], while *cis*- and *trans*-ferulates **46** and coumarates **47** inhibited phospholipase Cγ1 as well as the *in-vitro* proliferation of a number of tumor cell lines (see Table 1) [46]. Oleanane caffeates **48** and **49** both showed cytotoxic activity against MCF7, PC-3 and HCT-15 human tumor cell lines [55].

FRIEDELANE TRITERPENOIDS

Friedelane carboxylic acids **50** and **51** as well as dihydroxyfriedelin **52** exhibited *in-vitro* cytotoxic activity against A549 human lung carcinoma cells [56]. Maytenfolone-A (**53**) also showed *in-vitro* cytotoxic activity [57]. The nor-friedelane quinone-methide triterpenoids constitute a class of compounds generally having potent

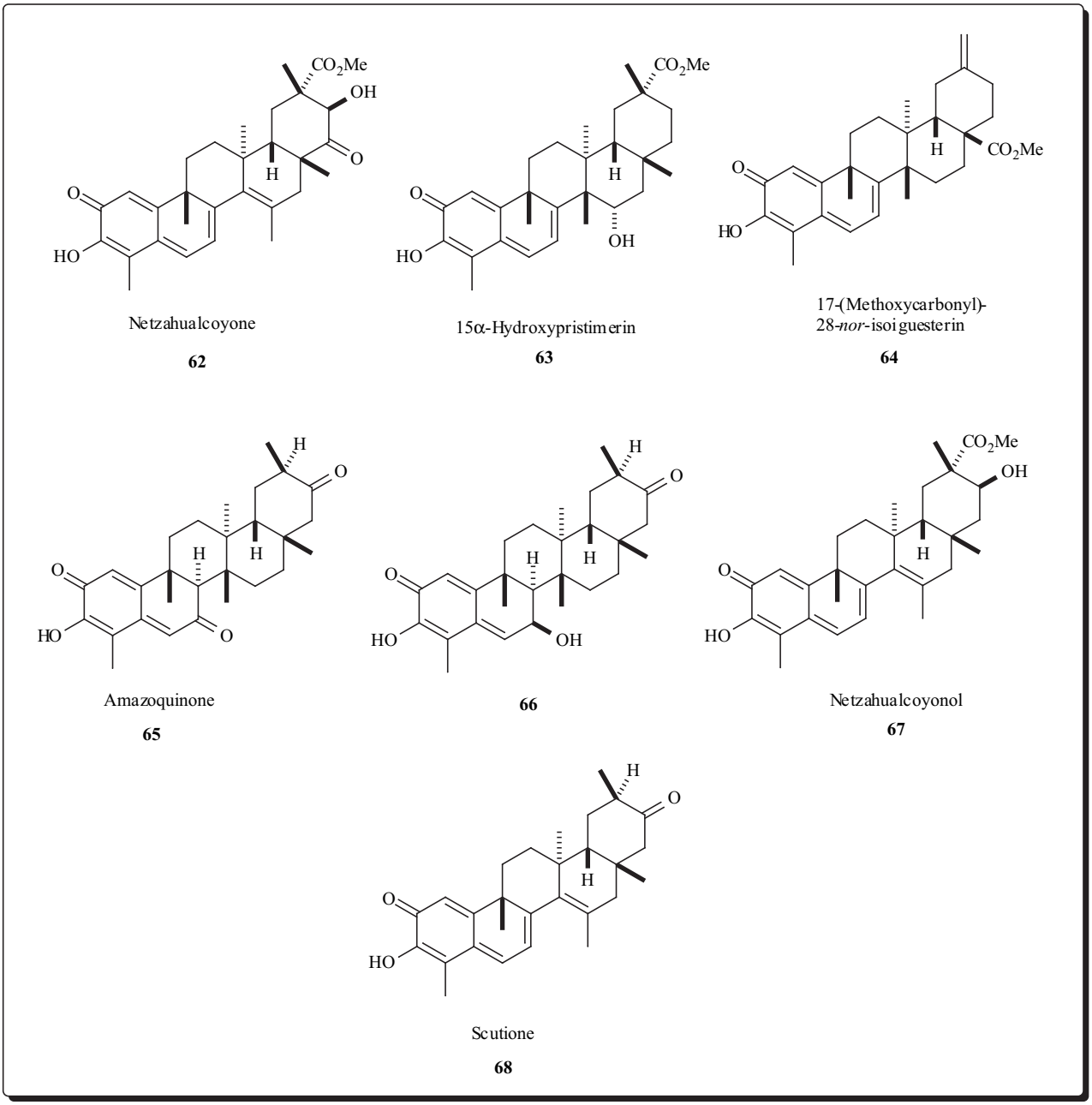


cytotoxic activity. The chemistry and biological activity of this class of compounds has been thoroughly reviewed by Gunatilaka [58], therefore only recent developments are included here. Tingenone (**54**) has demonstrated a broad-spectrum of cytotoxic activity on ten cell lines (see Table 1) [58-60] with L-1210 being the most susceptible [68].

Spectroscopic evidence for tingenone interacting with DNA [61] and molecular modeling studies support quasi-intercalation into DNA with concomitant nucleophilic addition of a purine base [62]. Pristimerin (**55**), celastrol (**56**), iguesterin (**57**), isoiguesterin (**58**), 22β-hydroxytingenone (**59**), 20-hydroxy-20-*epi*-tingenone (**60**), netzahualcoyondiol (**61**), and netzahualcoyone (**62**) have also exhibited broad-spectrum cytotoxic activity [58]. Recently, the quinone-methide triterpenoids 15α-hydroxypristimerin (**63**) [63], 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**64**) [64], amazoquinone (**65**) [65], 7β-hydroxydihydrotingenone (**66**) [65], netzahualcoyonol (**67**) [62], and scutione (**68**) [66], have been reported to be cytotoxic on a number of cell lines (see Table 1) with IC_{50} values ranging from 0.6 to 13 μ*M*.

A number of ring A aromatized derivatives of the quinone-methide group of triterpenoids have been isolated and characterized. These compounds (**69**-**75**) generally have lower cytotoxic activity compared to the quinone methides (see Table 1). It had previously been shown that aromatization of the A-ring in quinone-methide triterpenoids results in diminished bioactivity [67], for example, 6-oxotingenol (**69**) [68] is reported to be two orders of magnitude less cytotoxic than tingenone (**54**) on P-388, L-1210, and KB cells. Similarly, 7,8-dihydro-6-oxo-tingenol (**70**) [65], 3-methyl-6-oxotingenol (**71**) [68], and 3-methyl-22β,23-dihydroxy-6-oxotingenol (**72**) [68], were less toxic than tingenone while 6-oxopristimerol (**73**) [68] and demethylzeylasteral (**74**) [69] were less cytotoxic than pristimerin (**55**) on the cell lines tested. Demethylzeylasterone (**75**), however, has been shown to be an inhibitor of topoisomerase IIα [70].

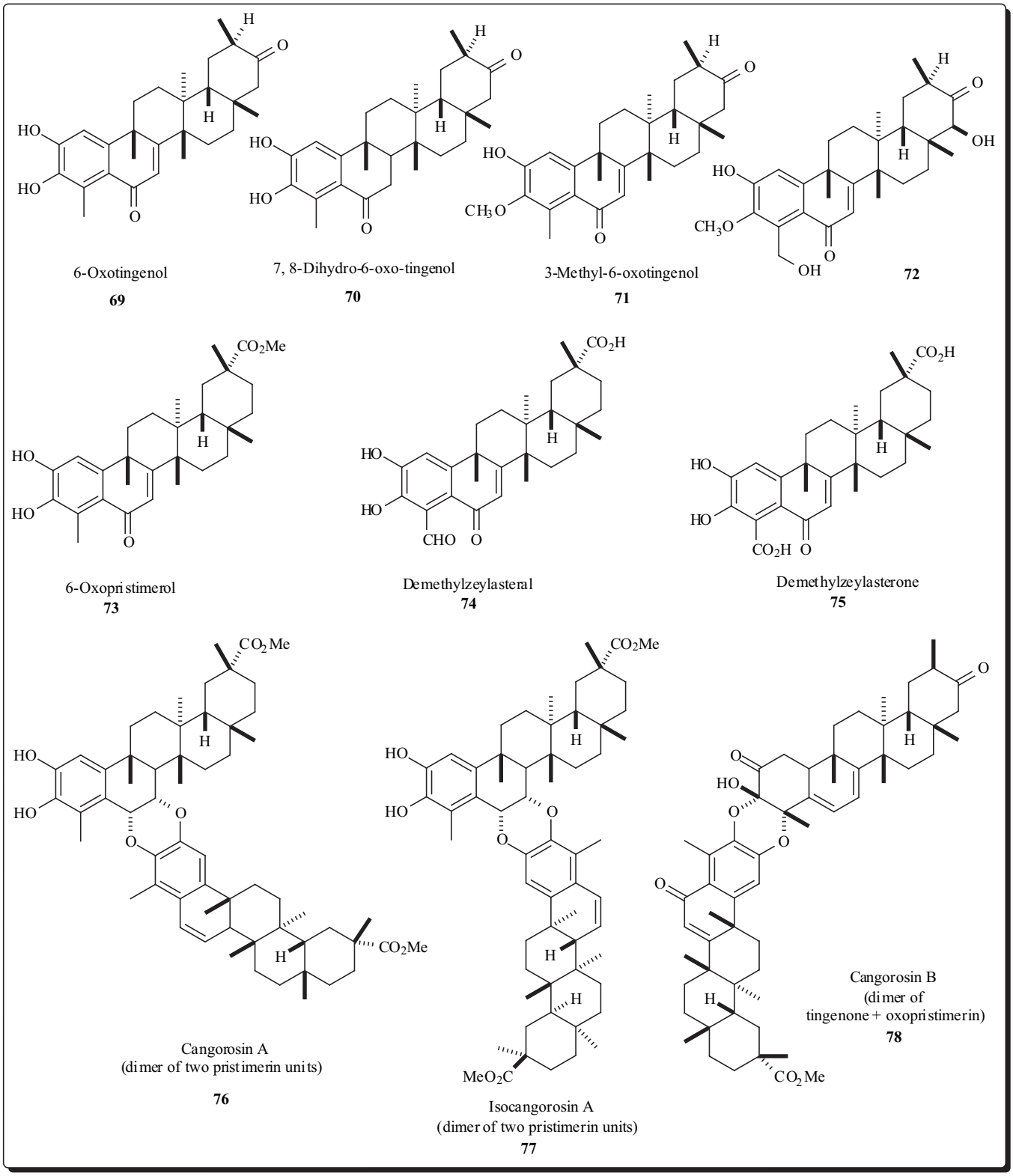
The cytotoxic activity of dimeric derivatives of quinone-methide triterpenoids is lower than the quinone methides themselves ([58] and references therein). Thus, cangorosin A

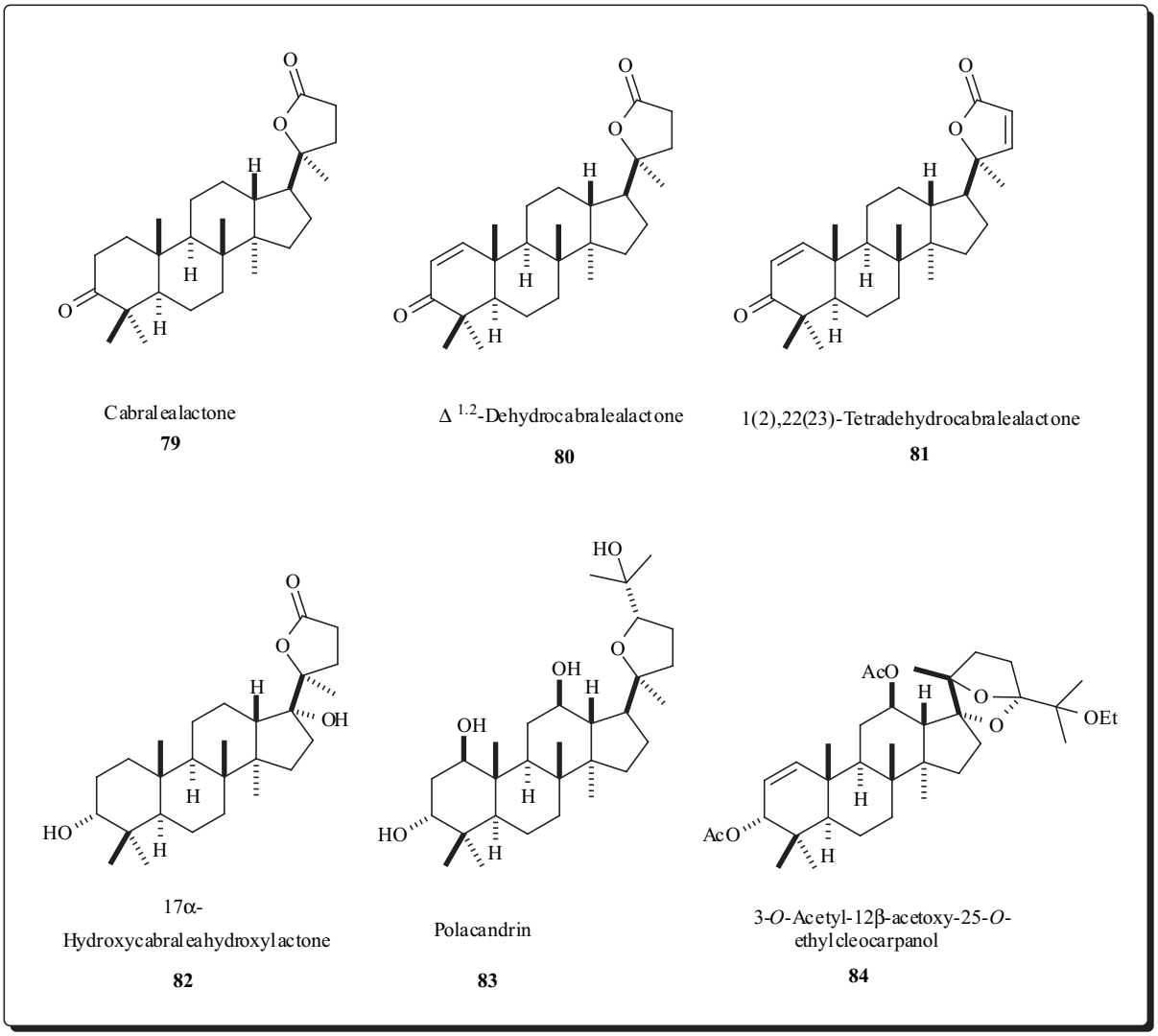


(**76**, a dimer of two pristimerin units), isocangorosin A (**77**, an alternative dimer of two pristimerin units), and cangorosin B (**78**, an adduct between tingenone and oxopristimerin) were non-cytotoxic toward P-388 cells (e.g., IC_{50} = 3.7 m*M* for **78**) [71]. Note that the structures of these materials have been subsequently revised [72]. The reduction in cytotoxic activity can be attributed to the loss of the quinone methide moiety upon dimerization.

DAMMARANE TRITERPENOIDS

Nagaya and co-workers [73] have isolated a number of cytotoxic dammarane triterpenoids from *Cleome africana* (Capparaceae). Thus, cabralealactone (**79**) and related compounds **80-82** exhibited *in-vitro* activity against the P-388 murine lymphocytic leukemia cell line. Polacandrin (**83**), from *Polanisia dodecandra*, also from the Capparaceae

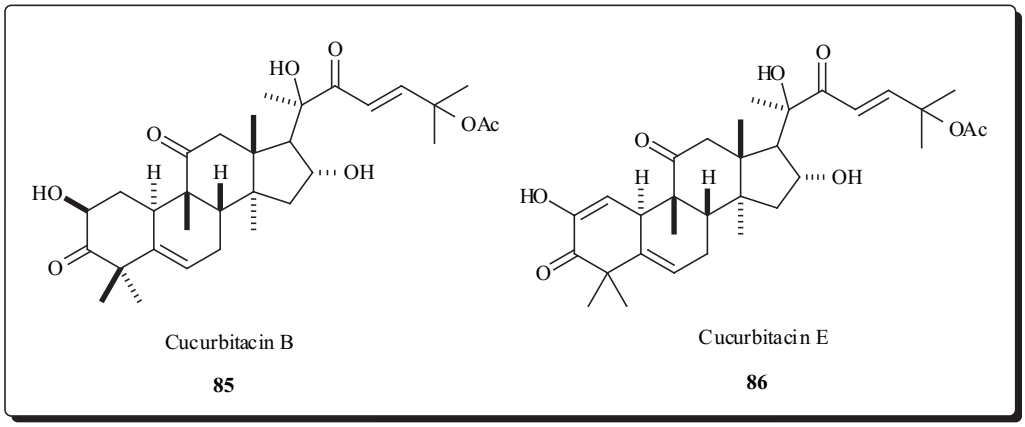


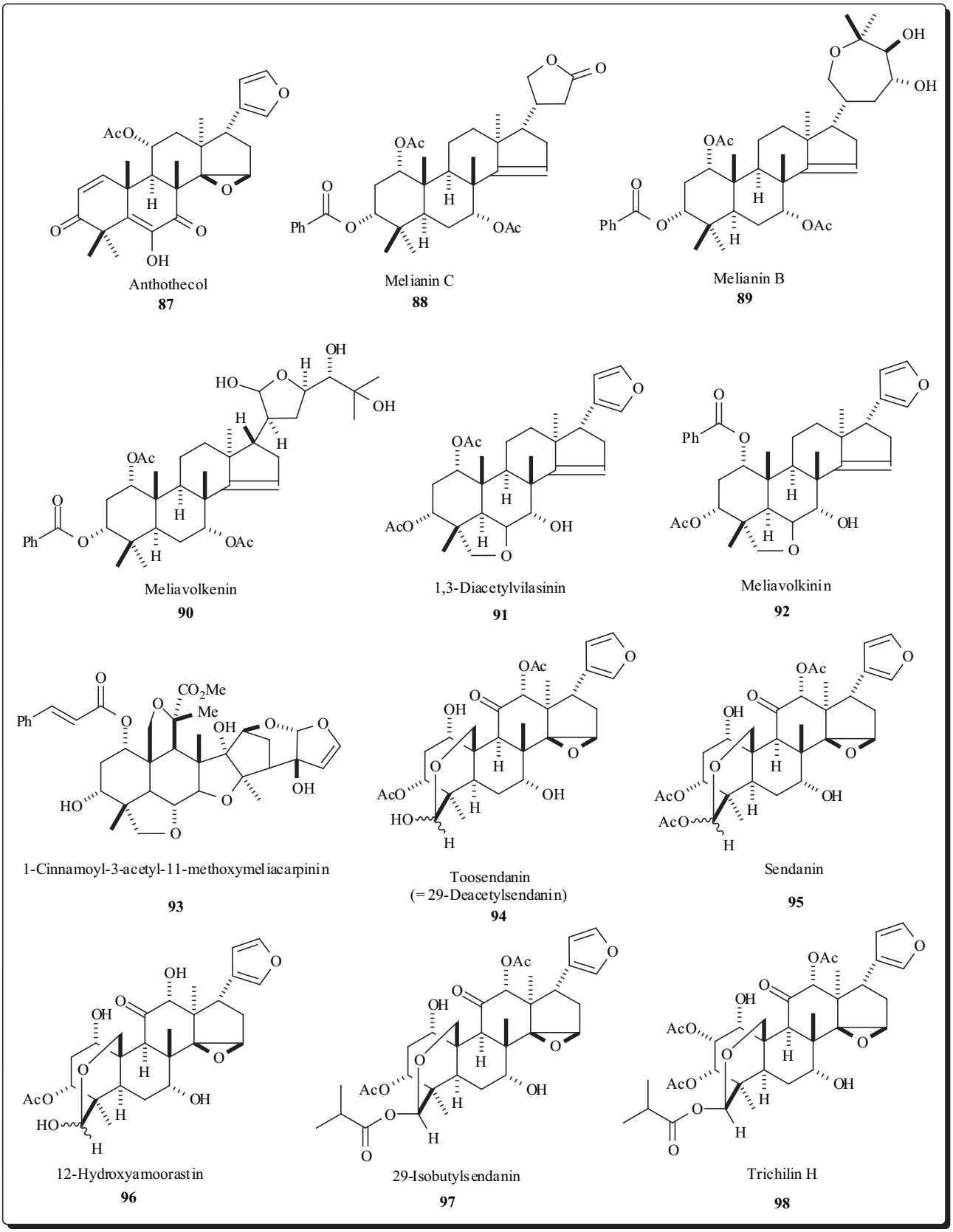


[74], and **84** from *Cleome africana* [73] were of similar magnitude of cytotoxicity on P-388 cells.

CUCURBITACINS

The cytotoxic activities of the cucurbitacins have been reported from the 1960's [25] and these represent the most cytotoxic triterpenoids (see Table 1), with cucurbitacin B (**85**) as one of the most potently cytotoxic on KB cells *in vitro* [75]. Cucurbitacin E (**86**) has been screened against the NCI panel of tumor-derived cells [76] and showed remarkable cytotoxicity against non-small lung (NCI-H226), melanoma (LOX IMVI), renal (A498 and CAKI-1), and breast (BT-549) tumor cells. Duncan and co-workers [77,78] have found cucurbitacin E to be cytotoxic to prostate cell lines, presumably by disruption of the actin cytoskeleton.





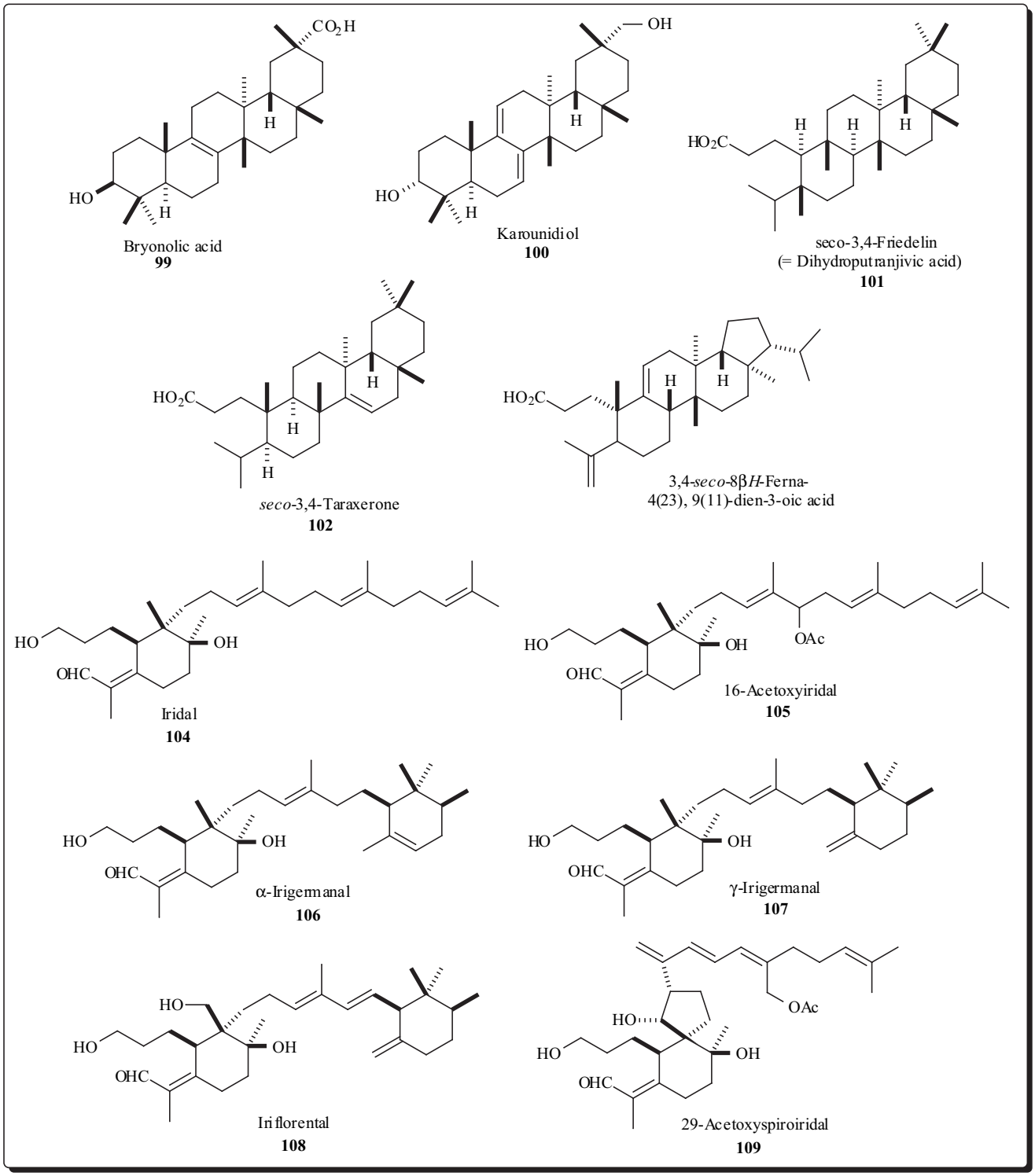
LIMONOIDS

The insect antifeedant and growth regulatory activities of limonoids have been reported [79,80], while some members of this class of compounds have been recognized for their cytotoxic activity as well. Anthothecol (**87**), for example, exhibited cytotoxic action against P-388 murine leukemia cells [81]. A number of cytotoxic compounds have been isolated from *Melia volkensii*, for example, melianin C (**88**), which shows selectivity cytotoxic activity against PC-3

prostate tumor cells, whereas melianin B (**89**) and meliavolkenin (**90**) demonstrated broad-spectrum cytotoxicity [82,83]. 1,3-Diacetylvilasinin (**91**) has been cytotoxic on A549, MCF7, PC-3, and HT-29 cells, whereas meliavolkinin (**92**) was selective for PC-3 cells [82]. The limonoids 1-cinnamoyl-3-acetyl-11-methoxymeliacarpinin (**93**) [84], toosendanin (**94**) [95], sendanin (**95**) [81], 12-hydroxyamoorastin (**96**) [81], and 29-isobutylsendanin (**97**) [85], were cytotoxic against P-388 cells *in vitro*, while toosendanin (**94**) and trichilin H (**98**) were also cytotoxic against KB cells [86].

MISCELLANEOUS TRITERPENOIDS

The multiflorane triterpenoid bryonolic acid (**99**) has shown *in-vitro* cytotoxic activity against B16 melanoma cells apparently by inducing apoptosis [87], while karounidiol (**100**) inhibited the proliferation of a number of cell lines, including the UO-31 human renal cancer cells [88]. Dihydroputranjivic acid (*seco*-3,4-friedelin, **101**) and the structurally related compound, *seco*-3,4-taraxerone (**102**), exhibited *in-vitro* cytotoxic activity against Hep G2 and A431 cells, an effect that is mediated by the inhibition of



topoisomerase II [89]. The *seco*-3,4-fernane triterpenoid **103** has also been shown to inhibit topoisomerase II [90]. The open A-ring seems to be an important structural feature conferring bioactivity to these compounds since neither friedelin nor taraxerone were cytotoxic. The iridals **104-109** have been reported to be of potent cytotoxicity against A2780 (human ovarian) and K562 (human leukemia) cancer cell lines [91].

SUMMARY

Antineoplastic natural products have been shown to inhibit tumor cell proliferation in a number of ways including, for example, interfering with cytoskeleton formation, preventing DNA replication, or interfering with signal transduction mechanisms. The cucurbitacin triterpenoids prevent proliferation of tumor cells by depolymerizing actin, thus interfering with the cytoskeleton. Quinone-methide triterpenoids interact with DNA in a quasi-intercalative manner followed by alkylation. Many of the cytotoxic triterpenoids described in the literature seem to exert their activity by interfering with DNA replication, typically by inhibition of DNA polymerase or inhibition of topoisomerase. For example, betulinic acid, ursolic acid, and oleanolic acid, all show inhibition of DNA polymerase β as well as inhibition of topoisomerases I and II. Clearly these compounds do not intercalate DNA; the binding of these compounds to the enzymes is unclear and warrants further study.

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