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Evaluation of *Aconitum* diterpenoid alkaloids as antiproliferative agents

Koji Wada[a,b], **Emika Ohkoshi**[b], **Yu Zhao**[b], **Masuo Goto**[b], **Susan L. Morris-Natschke**[b], and **Kuo-Hsiung Lee**[b,c,*]

[a]School of Pharmacy, Hokkaido Pharmaceutical University, 7-1, Katsuraoka-cho, Otaru 047-0264, Japan

[b]Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7568, USA

[c]Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan

Abstract

Little information has been reported on the antitumor effects of the diterpenoid alkaloid constituents of *Aconitum* plants, used in the herbal drug “bushi”. This study was aimed at determining the antitumor activities of *Aconitum* C_{19}-and C_{20}-diterpenoid alkaloids and synthetic derivatives against lung (A549), prostate (DU145), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KB-VIN) cancer cell lines. Newly synthesized C_{20}-diterpenoid alkaloid derivatives showed substantial suppressive effects against all human tumor cell lines tested. In contrast, natural and derivatized C_{19}-diterpenoid alkaloids showed only a slight or no effect. Most of the active compounds were hetisine-type C_{20}-diterpenoid alkaloids, specifically kobusine and pseudokobusine analogs with two different substitution patterns, C-11 and C-11,15. Notably, several C_{20}-diterpenoid alkaloids were more potent against multidrug-resistant KB subline KB-VIN cells. Pseudokobusine 11-3′-trifluoromethylbenzoate (**94**) is a possible promising new lead meriting additional evaluation against multidrug-resistant tumors.

Keywords

Diterpenoid alkaloids; Pseudokobusine; Antiproliferative agents

The genera *Aconitum*, *Consolida* and *Delphinium* (family Ranunculaceae) and the genus *Spiraea* (family Rosaceae) contain numerous diterpenoid alkaloids, which are classified

*Corresponding author. Tel.: +1 919 962 0065/0066; fax: +1 919 966 3893. khlee@unc.edu (K.-H. Lee).

Supplementary data

Physical and spectroscopic data for compounds **19–22, 62, 65, 67–70, 79, 93, 95, 106, 107**.

structurally as C_{18}-, C_{19}-, and C_{20}-diterpenoid alkaloids with the general structures and numbering systems shown in Figure 1.[1,2] *Aconitum* plants are used in “bushi”, an herbal traditional Chinese medicine prescribed to treat hypometabolism, dysuria, cardiac weakness, chills, neuralgia, gout, and certain rheumatic diseases.[3–5] Among the C_{19}-diterpenoid alkaloids, aconitine (**1**), jesaconitine (**3**), mesaconitine (**8**), and hypaconitine (**9**) exhibit particularly high toxicity, while the C_{20}-diterpenoid alkaloids lucidusculine (**37**), kobusine (**51**), pseudokobusine (**71**), and atisine are much less toxic. However, despite the extreme toxicities of some C_{19}-diterpenoid alkaloids, only two studies appeared in the literature in 2005 and 2006.[6,7] The first reported the antiproliferative activity of 8-*O*-azeloyl-14-benzoylaconine, an aconitine-type C_{19}-diterpenoid alkaloid,[6] and the second described the cytotoxic effects of various C_{19}-diterpenoid alkaloids against tumor cell lines.[7] Since 2007, many C_{19}- and C_{20}-diterpenoids as well as semisynthetic derivatives were evaluated for cytotoxicity by various assays, including cell growth, clonogenic, cell cycle distribution, and cell cycle-related, against four different human tumor cell lines, A172, A549, HeLa, and Raji.[8–12]

In an initial survey of the pharmacological effects of natural diterpenoid alkaloids and their synthetic derivatives, we tested 108 compounds for antiproliferative activity against four tumor cell lines, lung (A549), prostate (DU145), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KB-VIN) cancers. Here, we describe our results on the cytotoxic activities of these diterpenoid alkaloids and derivatives.

The C_{19}-diterpenoid alkaloids may be divided into six types: aconitine, lycoctonine pyro, lactone, 7,17-*seco*, and rearranged.[1,2] Most of the isolated C_{19}-diterpenoid alkaloids are aconitine- and lycoctonine-types. The C_{20}-diterpenoid alkaloids may be divided into ten types: atisine, denudatine, hetidine, hetisine, vakognavine, napelline, kusnezoline, racemulosine arcutine, and tricalysiamide.[1,2] Most of the isolated C_{20}-diterpenoid alkaloids are atisine-, hetisine-, and napelline-types. Table 1 lists the natural diterpenoid alkaloids and their source plant as well as the modified diterpenoid alkaloids generated for this study, and Figure 2 gives the structures of compounds **1**–**108**.

We tested 108 diterpenoid alkaloids for antiproliferative effects against four human tumor cell lines [lung carcinoma (A549), prostate carcinoma (DU145), nasopharyngeal (KB), and multi-drug resistant KB subline KB-VIN. Notably, we were interested in the antitumor activities against KB-VIN cells, because they overexpress drug transporter protein P-glycoprotein (P-gp), which effectively reduces intracellular drug concentration, especially of vinca and taxane alkaloids. The compounds included 24 natural (**1**–**6**, **8**–**10**, **12**–**15**, **17**, **23**–**25**, **28**–**32**, **34**, **36**) and 12 synthesized (**7**, **11**, **16**, **18**–**22**, **26**, **27**, **33**, **35**) C_{19}-diterpenoid alkaloids, as well as 10 natural (**37**–**39**, **41**, **48**–**51**, **71**, **85**) and 62 synthesized (**40**, **42**–**47**, **52**–**70**, **72**–**84**, **86**–**108**) C_{20}-diterpenoid alkaloids. Paclitaxel, a P-gp substrate anticancer agent, was used as an experimental control. The data are listed in Table 2. P-gp-overexpressing KB-VIN cells were over 100-fold resistant against paclitaxel, demonstrating that a lethal dose of paclitaxel may be required to be effective against MDR phenotype. The ratio of GI_{50} KB/GI_{50} KB-VIN demonstrated the efficacy of compound against KB-VIN. A549 (lung carcinoma), DU-145 (prostate cancer), and KB (epidermoid carcinoma) cell lines (ATCC) were supplied by Lineberger Comprehensive Cancer Center (UNC-CH).

Professor Y.-C. Cheng, Yale University, CT generously provided KBvin (vincristine-resistant KB subline). Cells were cultured in RPMI 1640 medium containing 25 mM HEPES and 2 mM L-glutamine (Mediatech), supplemented with 10% heat-inactivated fetal bovine serum (Hyclone), 100 IU penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Mediatech). KB-VIN cells were grown in media containing 100 nM vincristine and were cultured for 7–10 days without vincristine before experiments were performed. Cells were maintained in a humidified 5% CO_2 atmosphere at 37 °C, and passaged every 3–4 days. Cell viability was determined by the sulforhodamine B (SRB) colorimetric assay.[32] In brief, cells (3–5 × 10^3 cells/well) were seeded in 96-well plates containing various concentrations of samples or paclitaxel (PXL) as an assay control, and incubated for 72 h. At the end of the exposure period, the attached cells were fixed with 50% trichloroacetic acid for 30 min followed by staining with 0.04% SRB (Sigma Chemical Co.) for 30 min. The bound SRB was solubilized in 10 mM Tris-base and the absorbance was measured at 515 nm on a Microplate Reader ELx800 (Bio-Tek Instruments, Winooski, VT) with Gen5 software. All results were representative of three or more experiments.

All tested aconitine-type C_{19}-diterpenoid alkaloids, both the natural alkaloids (**1**–**6**, **8**–**10**, **12**–**14**) and synthetic analogs (**7**, **11**), were inactive (GI_{50} > 20 μM). Among the lycoctonine-type C_{19}-diterpenoid alkaloids, only compound **33**, esterified with 3-trifluoromethylbenzoyl at C-6, showed any, although very weak, cytotoxic activity with GI_{50} values of 12.6, 14.9, and 11.9 μM against DU145, KB, and KB-VIN cell lines, respectively. The remaining natural alkaloids (**15**, **17**, **23**–**25**, **28**–**32**, **34**, **36**) and synthetic analogs without a C-6 ester group (**16**, **18**–**22**, **26**, **27**, **35**) were inactive.

Among the C_{20}-diterpenoid alkaloids, both natural (**37**–**39**, **41**, **48**–**50**) and synthetic (**40**, **42**–**47**) napelline-type compounds, as well as hetidine-type synthetic analogs (**72**, **75**, **76**, **100**), were inactive. Among hetisine-type C_{20}-diterpenoid alkaloids, the natural parent alkaloids **51** and **71**, as well as a related synthetic analog (**108**) of **71**, were inactive against all four cancer cell lines. However, appropriate acylation, or in one case, etherification, of the two or three hydroxy groups in **51** and **71**, respectively, led to cytotoxic synthetic analogs (see Table 1).

Nine different groups were present on hydroxyls of the 11 hetisine-type C_{20}-diterpenoid alkaloids that exhibited average GI_{50} values of less than 10 μM: benzoyl (**56**, **79**), anisoyl (**58**), 4-nitrobenzoyl (**61**, **89**), 4-fluorobenzoyl (**69**), *trans*-3-trifluoromethylcinnamoyl (**70**), 3-nitrobenzoyl (**93**), 3-trifluoromethylbenzoyl (**94**), cinnamoyl (**98**), and trityl (**107**) groups. Nine additional compounds with six different ester groups exhibited average GI_{50} values between 10 and 20 μM: **78** (benzoyl), **81** (anisoyl), **59** (4-nitrobenzoyl), **64**, **96** (4-trifluoromethylbenzoyl), **65** (4-trifluoromethoxybenzoyl), **84**, **86**, and **87** (veratroyl). However, all compounds esterified with acetyl (**52**, **53**, **73**, **74**), 2-trifluoromethylbenzoate (**62**), propyl (**105**, **106**), pivaloyl (**101**), and nicotinoyl (**66**, **102**–**104**) groups, were inactive, regardless of the numbers or positions of the substitution. All six more potent (average GI_{50} < 10 μM) derivatives of **71** (**79**, **89**, **93**, **94**, **98**, **107**) had a free hydroxy group at C-6. Comparison of corresponding analogs of **71** and **51** showed that some compounds with a C-6 OH rather than H exhibited higher potency (compare **89** to **59**, **94** to **63**), although this pattern was not universal (compare **56** to **79**, **64** to **96**).

Among analogs of **51**, esterification of C-15 in addition to C-11 increased potency significantly (compare **59** to **61**) or even converted an inactive to an active compound (compare **54** to **56**, **57** to **58**, **67** to **69**). Consequently, all of the most analogs (**56**, **58**, **61**, **69**, **70**) of **51** were esterified at both C-11 and C-15. Among analogs of **71**, four C-11 mono-substituted compounds (**89**, **94**, **98**, **107**) and two C-11,15 diesterified compounds (**79**, **93**) exhibited average GI_{50} values of less than 10 μM. Certain C-11 (**81**, **84**, **96**), C-6,11 (**78**, **86**) and C-6,15 (**87**) esterified compounds were generally less potent, while all C-6 (**77**, **80**, **88. 97**) and C-15 (**73**, **85**, **90**, **99**, **103**, **105**) mono-substituted compounds, as well as the tri-substituted analog (**92**), were inactive. Thus, all of the more active (GI_{50} < 10 μM) hetisine-type compounds had an ester or ether group on the C-11 hydroxyl and were either 11,15-diester analogs of **51** (H at C-6) or 11-monoester/11,15-diester analogs of **71** (OH at C-6).

Striking observations from the data in Table 2 were the degree and comparative ratio of KB/KB-VIN potency. Eleven compounds (**56**, **58**, **61**, **69**, **70**, **79**, **89**, **93**, **94**, **98**, and **107**) were quite potent (GI_{50} < 10 μM) against KB-VIN. Indeed, compound **70** exhibited a significantly low GI_{50} value of 3.1 μM. The ratios of KB to KB-VIN (GI_{50} KB/GI_{50} KB-VIN) were greater than 0.73 for all active compounds, with many compounds displaying comparable potency against the two cell lines, in contrast with paclitaxel (ratio of 0.0067). C_{20}-Diterpenoid analogs **70**, **94**, and **107** showed over 1.3-fold selectivity with their greatest cytotoxic activity against KB-VIN (GI_{50} KB/GI_{50} KB-VIN: 1.3, 1.5 and 1.3, respectively).

In mechanism of action studies on selected diterpenoid alkaloids, the C_{20}-diterpenoid alkaloid derivatives **81** and **96** showed important suppressive effects against Raji cells. Further study indicated that **96** inhibited extracellular signal-regulated kinase phosphorylation but induced enhanced phosphoinositide 3 kinase phosphorylation, leading to accumulation of Raji cells in the G1 or sub G1 phase.[33] More investigation is certainly warranted.

In summary, we have synthesized acylated derivatives of various C_{19}- and C_{20}-diterpenoid alkaloids. Totally, 108 natural alkaloids and their derivatives were evaluated against four tumor cell lines. Eighty-seven compounds were non-toxic (GI_{50} > 20 μM), and ten compounds showed mild antiproliferative effects (GI_{50} = 10–20 μM). Except for **33**, all of the most active compounds were hetisine-type C_{20}-diterpenoid alkaloids with two different substitution patterns, C-11 and C-11,15. Compounds **56**, **58**, **61**, **69**, **70**, **79**, **89**, **93**, **94**, **98** and **107**, which are acylated or tritylated at the C-11 hydroxyl, exhibited the greatest potency over all four tested cell lines, including multidrug-resistant KB-VIN. These results demonstrate that modified hetisine-type C_{20}-diterpenoid alkaloids are not substrates of P-gp and are effective against multi-drug resistant tumors. These promising new lead compounds merit continued studies to evaluate their potential as antitumor agents, particularly with enhanced resistant tumor selectivity. In addition, our results from modification-based antitumor activity studies can be used for further development of anticancer drugs overcoming a multidrug-resistant phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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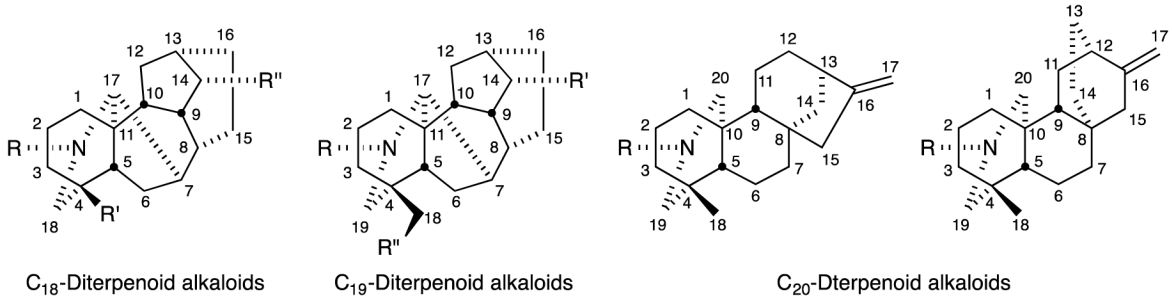
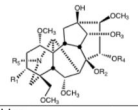
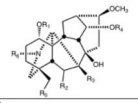


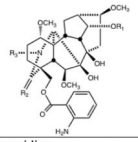
Figure 1.
General structures and numbering systems for C_{18}-, C_{19}-, and C_{20}-diterpenoid alkaloids.

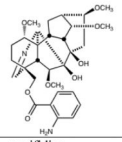


		R_1	R_2	R_3	R_4	R_5
1	aconitine	OH	Ac	Bz	H	Et
2	deoxyaconitine	H	Ac	Bz	H	Et
3	jesaconitine	OH	Ac	As	H	Et
4	deoxyjesaconitine	H	Ac	As	H	Et
5	aljesaconitine A	OH	Et	As	H	Et
6	see structure below					
7	3,15-diacetyljesaconitine	OAc	Ac	As	Ac	Et
8	mesaconitine	OH	Ac	Bz	H	Me
9	hypaconitine	H	Ac	Bz	H	Me
10	hokbusine A	OH	Me	Bz	H	Me
11	3-acetylmesaconitine	OAc	Ac	Bz	H	Me



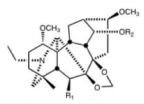
		R_1	R_2	R_3	R_4	R_5	R_6
12	neoline	H	α-OMe	H	H	OMe	Et
13	14-benzoylneoline	H	α-OMe	H	Bz	OMe	Et
14	karacoline	H	H	H	H	H	Et
15	delcosine	H	β-OMe	OH	H	OMe	Et
16	1-acetyldelcosine	Ac	β-OMe	OH	H	OMe	Et
17	14-acetyldelcosine	H	β-OMe	OH	Ac	OMe	Et
18	1,14-diacetyldelcosine	Ac	β-OMe	OH	Ac	OMe	Et
19	14-benzyldelcosine	H	β-OMe	OH	Bn	OMe	Et
20	1,14-di-(4-nitrobenzoyl)delcosine	NB	β-OMe	OH	NB	OMe	Et
21	1-(4-nitrobenzoyl)delcosine	NB	β-OMe	OH	H	OMe	Et
22	1-(4-trifluoromethylbenzoyl)delcosine	PFMB	β-OMe	OH	H	OMe	Et
23	see structure below						
24	delphinifoline	H	β-OH	OH	H	OMe	Et
25	14-acetylbrowniine	OMe	β-OMe	OH	Ac	OMe	Et
26	*N*-deethyldelsoline	H	β-OMe	OH	OMe	OMe	H
27	delsoline	H	β-OMe	OH	OMe	OMe	Et

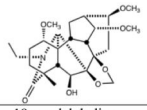




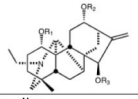
		R_1	R_2	R_3		
28	andersonidine	Ac	H_2	Et	**31**	pacifidine
29	pacifiline	Me	O	Et		

30	pacifinine	Me	O	H		

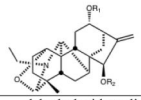




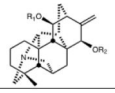
		R_1	R_2			
32	delpheline	OH	Me	**35**	19-oxodelpheline	
33	6-(3-trifluoromethylbenzoyl)delpheline	O-MFMB	Me			
34	pacinine	=O	Me			
36	yunnadelphinine	=O	H			



		R_1	R_2	R_3	
37	lucidusculine	H	H	Ac	
38	flavadine	H	H	Ac	*N*-oxide
39	12,15-diacetylluciculine	H	Ac	Ac	
40	1,12,15-triacetylluciculine	Ac	Ac	Ac	
41	1-acetylluciculine	Ac	H	H	
42	12-benzoylluciculine	H	Bz	H	
43	12-anisoylluciculine	H	As	H	
44	12-veratroylluciculine	H	Vr	H	
45	12-benzoyllucidsuculine	H	Bz	Ac	
46	1,12,15-tribenzoylluciculine	Bz	Bz	Bz	
47	12,15-dibenzoylluciculine	H	Bz	Bz	

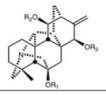


		R_1	R_2
48	dehydrolucidusculine	H	Ac
49	dehydroluciculine	H	H
50	12-acetyldehydroluciduscuiline	Ac	Ac

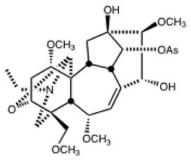


		R_1	R_2
51	kobusine	H	H
52	15-acetylkobusine	H	Ac
53	11,15-diacetylkobusine	Ac	Ac
54	11-benzoylkobusine	Bz	H
55	15-benzoylkobusine	H	Bz
56	11,15-dibenzoylkobusine	Bz	Bz
57	11-anisoylkobusine	As	H
58	11,15-dianisoylkobusine	As	As

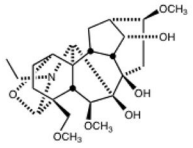
59	11-(4-nitrobenzoyl)kobusine	NB	H
60	15-(4-nitrobenzoyl)kobusine	H	NB
61	11,15-di-(4-nitrobenzoyl)kobusine	NB	NB
62	11-(2-trifluorometyhlbenzoyl)kobusine	OFMB	H
63	11-(3-trifluoromethylbenzoyl)kobusine	MFMB	H
64	11-(4-trifluoromethylbenzoyl)kobusine	PFMB	H
65	11-(4-trifluoromethoxybenzoyl)kobusine	FMTB	H
66	11-nicotinoylkobusine	Nt	H
67	11-(4-fluorobenzoyl)kobusine	FB	H
68	15-(4-fluorobenzoyl)kobusine	H	FB
69	11,15-di-(4-fluorobenzoyl)kobusine	FB	FB
70	11,15-di-(3-trifluoromethylcinnamoyl)kobusine	FMCM	FMCM



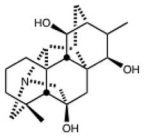
		R_1	R_2	R_3
71	pseudokobusine	H	H	H
73	15-acetylpseudokobusine	H	H	Ac
74	11,15-diacetylpseudokobusine	H	Ac	Ac
77	6-benzoylpseudokobusine	Bz	H	H
78	6,11-dibenzoylpseudokobusine	Bz	Bz	H
79	11,15-dibenzoylpseudokobusine	H	Bz	Bz
80	6-anisoylpseudokobusine	As	H	H
81	11-anisoylpseudokobusine	H	As	H
82	6,11-dianisoylpseudokobusine	As	As	H
83	6,15-dianisoylpseudokobusine	As	H	As
84	11-veratroylpseudokobusine	H	Vr	H
85	15-veratroylpseudokobusine	H	H	Vr
86	6,11-diveratroylpseudokobusine	Vr	Vr	H
87	6,15-diveratroylpseudokobusine	Vr	H	Vr
88	6-(4-nitrobenzoyl)pseudokobusine	NB	H	H
89	11-(4-nitrobenzoyl)pseudokobusine	H	NB	H
90	15-(4-nitrobenzoyl)pseudokobusine	H	H	NB
91	6,15-di-(4-nitrobenzoyl)pseudokobusine	NB	H	NB
92	6,11,15-tri-(4-nitrobenzoyl)pseudokobusine	NB	NB	NB
93	11,15-di-(3-nitrobenzoyl)pseudokobusine	H	MNB	MNB
94	11-(3-trifluoromethylbenzoyl)pseudokobusine	H	MFNB	H
95	6,11-di-(3-trifluoromethylbenzoyl)pseudokobusine	MFMB	MFMB	H
96	11-(4-trifluoromethylbenzoyl)pseudokobusine	H	PFMB	H
97	6-cinnamoylpseudokobusine	Cm	H	H
98	11-cinnamoylpseudokobusine	H	Cm	H
99	15-cinnamoylpseudokobusine	H	H	Cm
101	11-pivaloylpseudokobusine	H	Pv	H
102	11-nicotinoylpseudokobusine	H	Nt	H
103	15-nicotinoylpseudokobusine	H	H	Nt
104	11,15-dinicotinoylpseudokobusine	H	Nt	Nt
105	15-propionylpseudokobusine	H	H	Pr
106	11,15-dipropionylpseudokobusine	H	Pr	Pr
107	11-tritylpseudokobusine	H	Tr	H



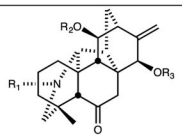
6 secojesaconitine



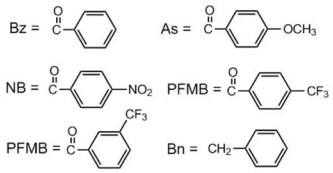
23 18-methoxygadesine



108 dihydropseudokobusine



		R_1	R_2	R_3
72	*N*-benzyl-*N*,6-seco-6-dehydropseudokobusine	Bn	H	H
75	*N*,11,15-triacetyl-*N*,6-seco-6-dehydropseudokobusine	Ac	Ac	Ac
76	*N*-acetyl-*N*,6-seco-6-dehydropseudokobusine	Ac	H	H
100	*N*-cinnamoyl-*N*,6-seco-6-dehydropseudokobusine	Cm	H	H



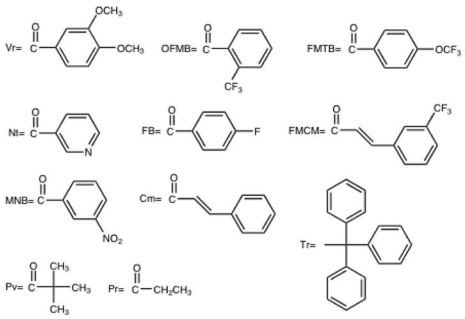


Figure 2.
Structures of compounds **1**–**108**

Table 1

Identities, types, and sources of diterpenoid alkaloids and derivatives

Diterpenoid Alkaloid Type/Class	Natural Cmpds	Plant Source	References	Diterpenoid Alkaloid Type/Class	Modified Cmpds	References
Aconitine-type C_{19}	**1–6, 8–10, 12–14**	*Aconitum japonicum* Thunb. roots	13–17	Aconitine-type C_{19}	**7, 11**	14, 18
Lycoctonine-type C_{19}	**15, 17, 23–25**	*A. yesoense* var. *macroyesoense* (Nakai) Tamura roots	19–22	Lycoctonine-type C_{19}	**16, 18–22, 26, 27**	5, 10, 23, 24
Lycoctonine-type C_{19}	**28–32, 34, 36**	*Delphinium elatum* cv. Pacific Giant seeds	25, 26	Lycoctonine-type C_{19}	**33, 35**	12
Napelline- & hetisine-type C_{20}	**37–39, 41, 48–51, 71, 85**	*A. yesoense* var. *macroyesoense* roots	19, 21, 22	Napelline-type C_{20}	**40, 42–47**	10, 19, 27
				Hetisine-type C_{20}-analogs of **51**	**52–70**	10, 11, 28, 29
				Hetisine-type C_{20}-analogs of **71**	**72–84, 86–108**	10, 19, 28–31

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Table 2

Cytotoxic activity data for diterpenoid alkaloids and derivatives [a,b,c]

Position/Substituent				GI_{50} (μM)				
Cmpd	**6**	**11**	**15**	**A549**	**DU145**	**KB**	**KB-VIN**	**KB/KB-VIN ratio**
56	H	OBz	OBz	8.4	9.3	6.0	7.5	0.80
58	H	OAs	OAs	6.7	7.1	5.3	5.2	1.0
61	H	ONB	ONB	6.9	7.0	5.3	5.5	0.96
69	H	OFB	OFB	8.1	6.8	5.2	7.1	0.73
70	H	OFMCM	OFMCM	5.5	6.2	4.1	3.1	1.3
79	OH	OBz	OBz	8.8	7.6	5.2	6.3	0.82
89	OH	ONB	OH	5.8	7.2	6.4	6.4	1.0
93	OH	OMNB	OMNB	5.0	5.2	5.6	5.6	0.99
94	OH	OMFMB	OH	6.8	7.7	8.9	6.2	1.5
98	OH	OCm	OH	8.4	6.5	7.0	6.4	1.1
107	OH	OTr	OH	6.4	6.0	6.6	5.2	1.3
paclitaxel				0.0071	0.0057	0.0064	0.95	0.0067

[a] Compounds **33**, **59**, **64**, **65**, **78**, **81**, **84**, **86**, **87**, **96** exhibited average GI_{50} values > 10 but < 20 μM. Compounds **1**–**32**, **34**–**55**, **57**, **60**, **62**, **63**, **66**–**68**, **71**–**77**, **80**, **82**, **83**, **85**, **88**, **90**–**92**, **95**, **97**, **99**–**106**, **108** were inactive (GI_{50} > 20 μM) against all four cancer cell lines.

[b] Cytotoxicity as GI_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 515 nm relative to untreated cells using sulforhodamine B assay.

[c] Lung carcinoma (A549), prostate carcinoma (DU145), nasopharyngeal (KB), and multidrug-resistant KB subline expressing P-glycoprotein (KB-VIN).