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Two decades of advances in diterpenoid alkaloids with cytotoxicity activities

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The important pharmacological activities and structural complexity of diterpenoid alkaloids have long stimulated strong scientific interest; some of these naturally abundant compounds have been reported to be highly promising for treating cancer. From 2008 to 2018, the cytotoxicity activities of more than 250 diterpenoid alkaloids were tested against several cancer cell lines. This review focuses on the progress of diterpenoid alkaloids with different structures derived from Ranunculaceae plants and some of their derivatives with potential anticancer activities. Then, we discuss the application prospects and development of active diterpenoid alkaloids.

1. Introduction

Throughout medical history, the majority of drugs used in cancer chemotherapy have been categorized as plant alkaloids, alkylating agents, antimetabolites, antibiotics, topoisomerase inhibitors, monoclonal antibodies, and other antitumor agents.[1–9] Due to their important pharmacological activities and structural complexity, the phytochemistry, synthesis, and medicinal chemistry of diterpenoid alkaloids have stimulated strong scientific interest for a long time; however, little information on their cytotoxic properties has been reported.

Recently, numerous diterpenoid alkaloids have been isolated from various species of *Aconitum*, *Consolida*, and *Delphinium* (Ranunculaceae); they are classified according to their chemical structures as C_{18}-, C_{19}-, or C_{20}-diterpenoid alkaloids (Fig. 1).[10,11] Their therapeutic potentials are being extensively studied: multiple data on their analgesic and anti-inflammatory activities, their effects on the central nervous system, and their arrhythmogenic, antiarrhythmic, antiparasite and anticancer properties are being continuously reported. These compounds still represent an active area of research. Accordingly, a number of reviews on various aspects of diterpenoid alkaloids have been published in the past decades, such as Pelletier's review in 1999,[12] Wang and Liang's contributions in 2002 and 2009,[13,14] and Wang's latest work in 2010.[10,11] Those reviews mostly cover the classification, distribution and occurrence, biosynthesis, spectroscopy, chemical reactions and stereochemistry, and pharmacological activity of diterpenoid alkaloids.

However, fewer reviews have focused on the cytotoxicity of these compounds. In this paper, we will review advances in the cytotoxicity activities of diterpenoid alkaloids in the past two decades (from 2008 to 2018) in order to determine promising areas for therapeutic applications.

The first reported antiproliferative activity of 8-*O*-azeloyl-14-benzoylaconine (**1**) (Fig. 2), an aconitine-type C_{19}-diterpenoid alkaloid (IC_{50} values against HCT-15, A549 and MCF-7 ranging from 10 to 20 μM), was published in 2005.[15] In the same year, a second report on a C_{18}-derivative, lappaconitine hydrobromide (**2**), showed efficient antitumor effects in mice, with inhibition rates ranging from 11.20% to 53.08% for liver tumor growth and from 29.81% to 53.96% for S180 tumor growth.[16] A third report in 2006 described the cytotoxic effects of various C_{19}-diterpenoid alkaloids against tumor cell lines,[17] among which neoline (**3**), 8-*O*-methylcolumbianine (**4**), 1,14-diacetylcardiopetaline (**5**), 18-*O*-demethylpubescenine (**6**), 14-deacetylpubescenine (**7**), pubescenine (**8**), 14-deacetylajadine (**9**), lycoctonine (**10**), browniine (**11**), delphatine (**12**), dehydrotakaosamine (**13**), and ajadelphinine (**14**) (Fig. 2) exhibited selective cytotoxicity to cancerous *versus* non-cancerous cells. In an additional report by Kashiwakura *et al.* in 2007, the cytotoxicities of ten C_{19}-diterpenoid alkaloids and thirty-three C_{20}-diterpenoid alkaloids against A172 (human malignant glioma cells) were examined; three C_{20}-diterpenoid alkaloids (**15a–c**) were found to be the most potent cytotoxic agents. The other compounds had very weak activities.[18] Since 2007, the cytotoxicities of many C_{19}- and C_{20}-diterpenoid alkaloids as well as their semisynthetic derivatives against different human tumor cell lines have been evaluated by various assays, including cell growth, cell cycle distribution, and cell cycle-related assays.[19–45]

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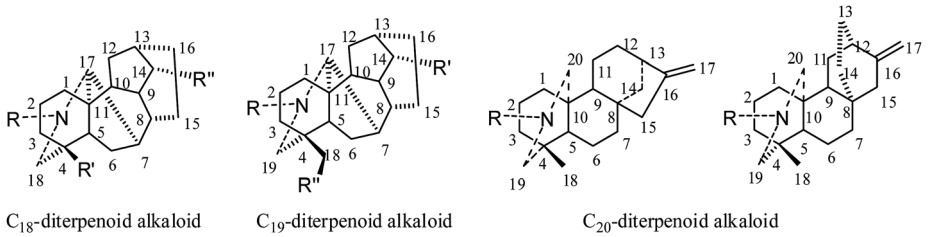


Fig. 1 General structures and numbering systems for C_{18}-, C_{19}-, and C_{20}-diterpenoid alkaloids.

2. Progress in cytotoxicity research of diterpenoid alkaloids

2.1 C_{18}-diterpenoid alkaloids with cytotoxicity activities

Lappaconitine (**16**) (Fig. 3), a typical C_{18}-diterpenoid alkaloid, is commonly used as postoperative analgesia and relief for clinical cancer pain as a non-addictive analgesic.

After the first report of lappaconitine hydrobromide in 2005, Xiong and coworkers observed its dose-dependent inhibition of the proliferation of A549 human non-small cell lung cancer cells in 2010 and 2014.[19,20] With increasing lappaconitine concentration, the proportion of A549 cells in G1 + G0 phase increased gradually and the proportion in S and G2 + M phases decreased; the apoptosis rate increased with down-regulated expression of cyclin E1. Also, the expression of VEGF-A was inhibited, and a combination of lappaconitine and oxaliplatin arrested cells in G1/G0 phase and inhibited the expression of cyclin E1.[20]

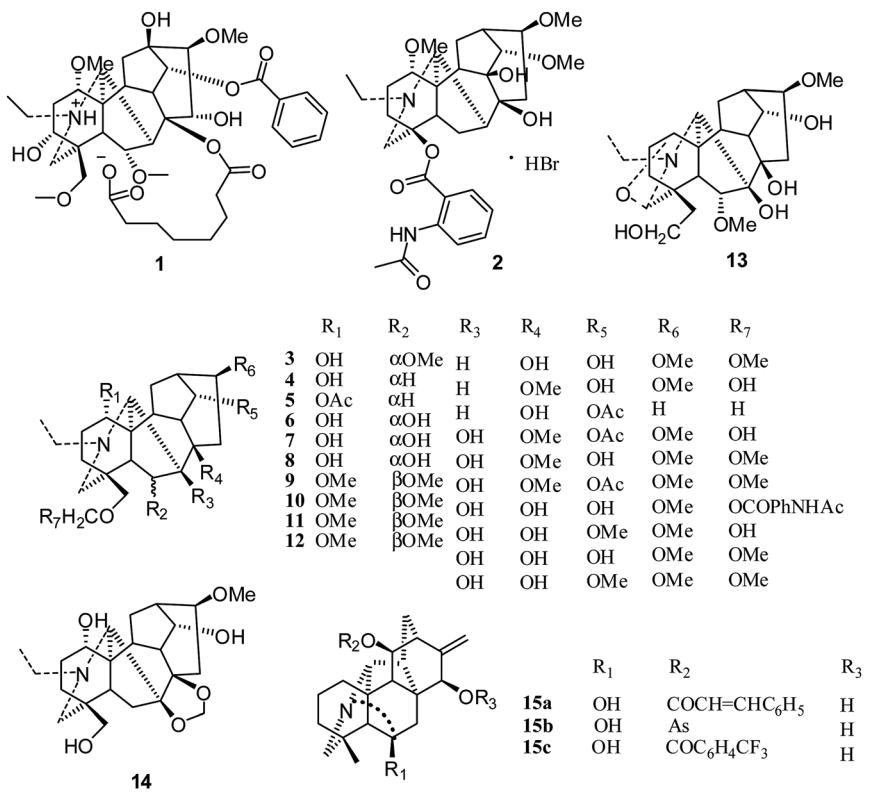
In 2018, Liang's group reported nineteen lappaconitine azacinnamic acid derivatives (**17**) (Fig. 3) with cytotoxicity activities;[21] only **17a–c** exhibited inhibited effects on tumor cells, with equal or better activity than 5-fluorouracil against the human prostate cancer cell line (PC-3), human gastric adenocarcinoma cell line (SGC-7901) and human lung cancer cell line (A-549).

2.2 C_{19}-Diterpenoid alkaloids with cytotoxicity activities

The C_{19}-diterpenoid alkaloids are usually divided into six types: lycoctonine, aconitine, pyro, lactone, 7,17-seco, and rearranged.[10,11] Most of the isolated C_{19}-diterpenoid alkaloids are lycoctonine- and aconitine-type.

2.2.1 Progress in the cytotoxicity research of C_{19}-diterpenoid alkaloids

Lycoctonine-type. In 2010, Gao and coworkers reported the cytotoxic activities of delpheline (**18**), delbrunine (**19**), and delectinine (**20**) against the MCF-7 and A549 cell lines (Fig. 4).[22]



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
3	OH	αOMe	H	OH	OH	OMe	OMe
4	OH	αH	H	OMe	OH	OMe	OH
5	OAc	αH	H	OH	OAc	H	H
6	OH	αOH	OH	OMe	OAc	OMe	OH
7	OH	αOH	OH	OMe	OH	OMe	OMe
8	OH	αOH	OH	OMe	OAc	OMe	OMe
9	OMe	βOMe	OH	OMe	OH	OMe	OCOPhNHAc
10	OMe	βOMe	OH	OH	OH	OMe	OH
11	OMe	βOMe	OH	OH	OMe	OMe	OMe
12	OMe	βOMe	OH	OH	OH	OMe	OMe

	R_1	R_2	R_3
15a	OH	$COCH{=}CHC_6H_5$	H
15b	OH	As	H
15c	OH	$COC_6H_4CF_3$	H

Fig. 2 Early cytotoxicity studies of diterpenoid alkaloids (before 2008).

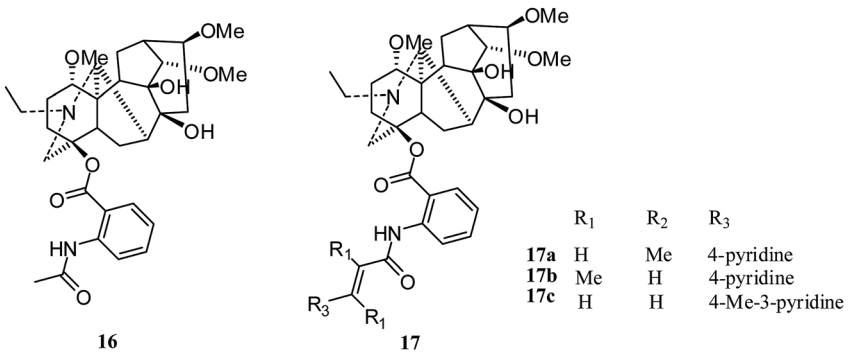


Fig. 3 Lappaconitine and its derivatives (16–17) with cytotoxicity activities.

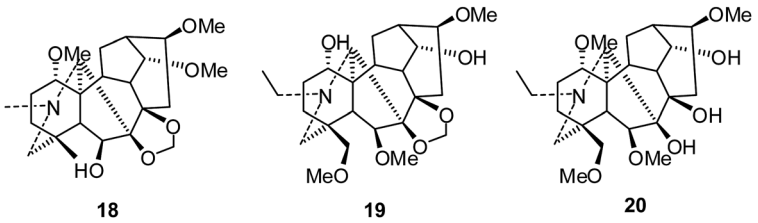


Fig. 4 The structures of C_{19}-diterpenoid alkaloids 18–20.

Compound **19** showed moderate activities against both cell lines, with IC_{50} values of 16.5 and 10.6 μM, respectively; meanwhile, compound **18** was only active against MCF-7 cells (IC_{50} 17.3 μM). In contrast, compound **20** was inactive for both tumor cell lines (IC_{50} > 50 μM).

Meanwhile, Lee's group synthesized twenty-four new derivatives (**21–47**) (Fig. 5 and 6) of delpheline (**18**) and evaluated their cytotoxic activities against lung (A549), prostate (DU145), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KB-VIN) cancer cell lines. Acylation of C-6 was found to be critical for cytotoxic activity.[27] Compounds **31**, **36**, **37**, and **39** displayed the greatest potency to the four tested cell lines. Compounds **31**, **32**, **35**, and **38** also exhibited greater inhibitory activities against drug-resistant KB-VIN cells (2.15 to 2.57 fold)

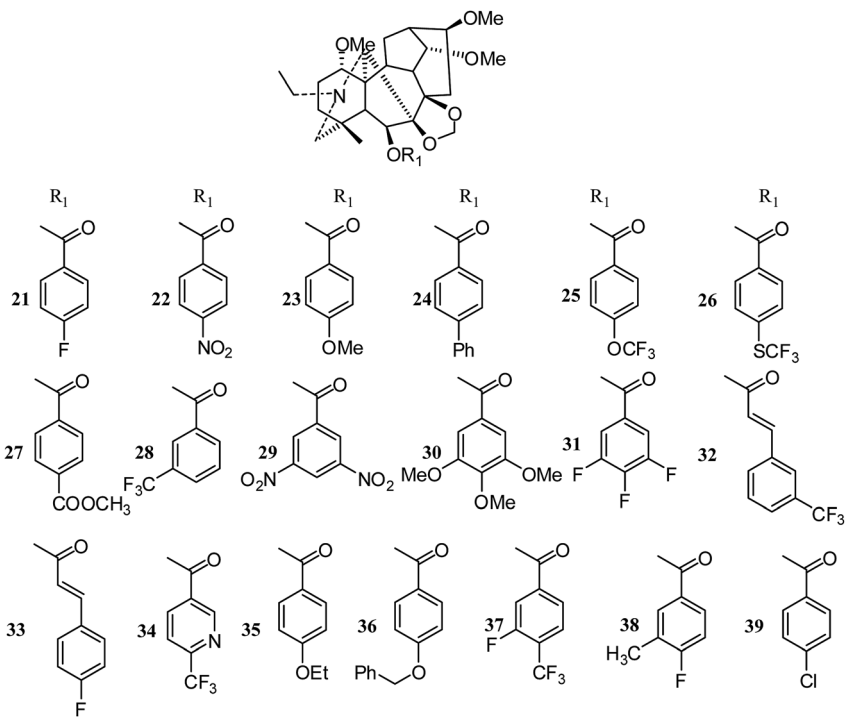


Fig. 5 New analogues (21–39) of delpheline (18) by Lee's group (I).

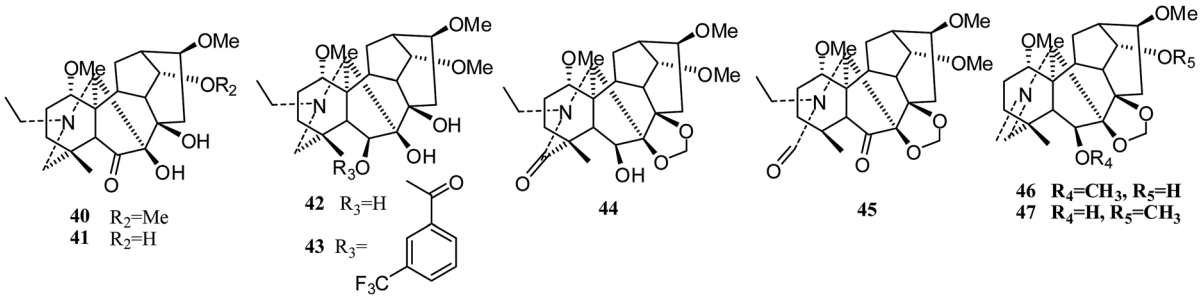
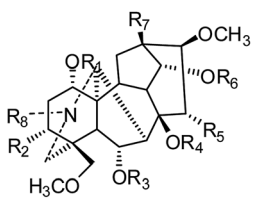


Fig. 6 New analogues (40–47) of delpheline (18) by Lee's group (II).

than the parental KB cells. Moreover, compounds **21**, **22**, **24**, **25**, **28**, **29**, and **32** showed tumor-selective activities, while others showed inactive potency against the four cell lines. Lee and coworkers also isolated three new delpheline analogues (**45–47**) (Fig. 6) from *Delphinium elatum* in 2015; these showed inactivity against the A549, DU145, KB, and KB-VIN cancer cell lines.[28] Furthermore, Lee *et al.* tested the antiproliferative activities of 108 diterpenoid alkaloids against four tumor cell lines (A549, DU145, KB and KB-VIN); thirty-six of these were C_{19}-diterpenoid alkaloids, including neoline (**3**), delpheline (**18**), 6-(3-trifluoromethylbenzoyl)-delpheline (**28**), aconitine (**48**), deoxyaconitine (**52**), hypaconitine (**53**), and mesaconitine (**54**).[25] However, only **28** exhibited moderately average IC_{50} values (10 to 20 μM).[29]

Aconitine-type. In 2012, eight C_{19}-diterpenoid alkaloids, aconitine (**48**), chasmanine (**49**), crassicauline A (**50**), oxonitine (**51**), deoxyaconitine (**52**), hypaconitine (**53**), mesaconitine (**54**), and senbusine A (**55**) (Fig. 7), were tested against several cell lines by the MTT method;[23] compounds **18** and **20–25** exhibited moderate to strong cytotoxic activities against the HCT8, MCF7 and HePG2 cell lines. Among these, aconitine (**48**), oxonitine (**51**) and deoxyaconitine (**52**) showed the strongest cytotoxic activities against the HCT8, MCF7 and HePG2 cancer cell lines, respectively. Moreover, in an earlier report, the reversal of multidrug resistance of aconitine (**48**) was evaluated in drug-resistant human oral squamous cell carcinoma (KBv200); it showed a slight inhibitory effect on the growth of KBv200 (IC_{50} = 224.91 μg ml^{-1}). However, it was considered to be a multidrug resistant reversing agent for increasing the sensitivity of vincristine to kill tumor cells.[24]



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
48	Me	OH	Me	Ac	OH	Bz	OH	CH_2CH_3
49	Me	H	Me	H	H	H	H	CH_2CH_3
50	Me	H	Me	Ac	H	As	OH	CH_2CH_3
51	Me	H	Me	Ac	OH	Bz	OH	CHO
52	Me	H	Me	Ac	OH	Bz	OH	CH_2CH_3
53	Me	H	Me	Ac	OH	Bz	OH	CH_3
54	Me	H	Me	Ac	OH	Bz	OH	CH_3
55	H	H	H	H	H	H	H	CH_2CH_3

Fig. 7 The structures of C_{19}-diterpenoid alkaloids 48–55.

In the same year, a series of mono-[*O*-(14-benzoylaconine-8-yl)]esters (**56a–h**) (Fig. 8), built from the 8-*O*-azeloyl-14-benzoylaconine scaffold and differing in the lengths of their alkyl linker chains, were synthesized and evaluated against a panel of human tumor cell lines: A-549, MCF-7 and HCT-15.[25] The results showed that none of the mono-[*O*-(14-benzoylaconine-8-yl)]esters displayed *in vitro* cytotoxicity activity (IC_{50} > 60 μM).

In 2014, three C_{19}-diterpenoid alkaloids, **57a–c** (Fig. 8), were isolated from *Aconitum taipeicum* by Guo's group, and their cytotoxicities against HL-60 and K562 cells were assayed; **57a** exhibited stronger cell growth inhibition than adriamycin.[30] Further study showed that **57a** inhibited the proliferation of HepG2 cells in a dose- and time-dependent manner and also inhibited the invasiveness of HepG2 cells. It also induced significant apoptosis of tumor cells at high dosages.[31]

After that, in 2017, Shen's group isolated three new C_{19}-diterpenoid alkaloids; only 15-hydroxyldelphisine (**58**) (Fig. 9) showed inhibition of the cancer cell line SK-OV-3 (IC_{50} 43.78 μM).[32] Later in 2017, Liang and coworkers reported the cytotoxic activities of three lipo-alkaloids (**59–61**) (Fig. 9) from *Aconitum sinchiangense* W. T. Wang; **59** and **60** showed significant cytotoxicity activities against tumor cells (HL-60, A-549, SMCC-7721, MCF-7 and SW480), with IC_{50} values comparable to that of cisplatin.[33,34]

2.2.2 The structure–cytotoxicity activity relationships of C_{19}-diterpenoid alkaloids. From 2008 to 2018, more than 100 C_{19}-diterpenoid alkaloids were studied for their cytotoxicity activities. In general, many of the natural and derivatized C_{19}-diterpenoid alkaloids showed only slight or no effects. Also, no detailed SAR study has yet been reported.

According to their early report in 2005, Robert *et al.* determined that the C-8 group may contribute to enhanced cytotoxic activity.[15] In 2007, although Kashiwakura *et al.* did not obtain C_{19}-diterpenoid alkaloids with high cytotoxic activities, they found that slight growth inhibitory activities were effected in the presence of a hydroxyl group (ether group or methoxy) or an N–H group (*N*-ethyl) substituent at C-1.[18] Then, from the present reports, Gao *et al.* concluded that the number of ester groups of alkaloids has an extraordinarily important influence on their cytotoxicity.[23] Obviously, the cytotoxicities of the C_{19}-

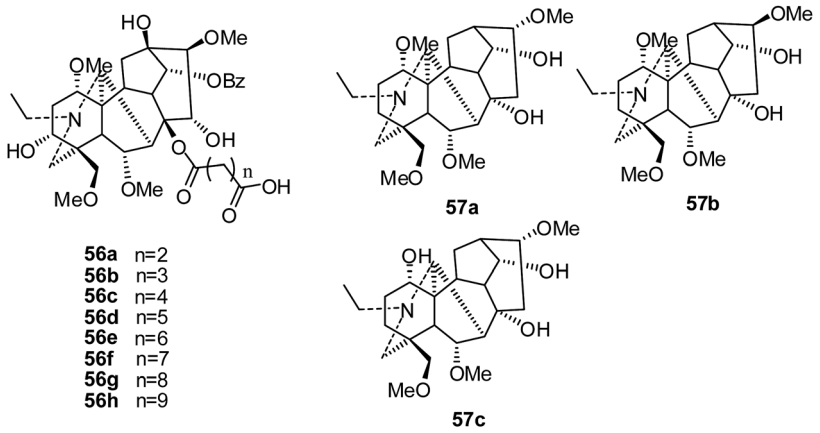


Fig. 8 The structures of C_{19}-diterpenoid alkaloids **56–57**.

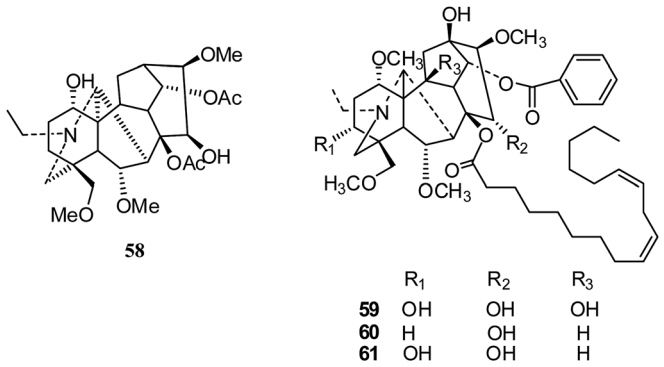


Fig. 9 The structures of C_{19}-diterpenoid alkaloids **58–61**.

diterpenoid alkaloids containing two ester groups, such as aconitine (**48**), crassicauline A (**50**), oxonitine (**51**), deoxyaconitine (**52**), hypaconitine (**53**) and mesaconitine (**54**), were markedly stronger than those of chasmanine (**49**) and senbusine A (**55**), which have no ester group substituents. From Lee's work, 6-acylation appears to be critical for producing cytotoxic activity.[27] According to Liang's work, the hydroxyl group at C-3 and the long-chain ester group at C-8 may increase cytotoxic activity.[33] Thus, in conclusion, the number and position of the hydroxyl and ester groups as well as the variety of ester groups require further study to identify more potent antitumor C_{19}-diterpenoid alkaloids. Specially, substitutions at C-1, C-3, C-6, and C-8 should be paid more attention.

2.2.3 The cytotoxicity mechanism of C_{19}-diterpenoid alkaloids. A few reports are available that study the cytotoxicity mechanisms of C_{19}-diterpenoid alkaloids. An earlier report on the study of aconitine (**48**) by immunohistochemistry and gene chip technology showed that it could downregulate the expression of protein Pgp and change the expression of the Mdr1 gene by affecting apoptosis-related genes and the mitogen-activated protein kinase (MAPK) signal transduction system, thereby ultimately reversing drug resistance.[35,36]

In 2014, Guo and co-workers found that **57a** blocked the cell cycle at the G1/S phase; further mechanism studies revealed that it may upregulate the protein expression of Bax and caspase-3 and downregulate the protein expression of Bcl-2 and CCND1.[31]

2.3 C_{20}-Diterpenoid alkaloids with cytotoxicity activities

The C_{20}-diterpenoid alkaloids are usually divided into four classes (atisane, kaurene, rearranged and bisditerpenoid classes) and nineteen types: atisine, denudatine, spireine, hetidine, cardionidine, albovionitine, hetisine, vakognavine, veatchine, napelline, anopterine, delnudine, kusnesoline, racemulosine, atisine–hetidine, rearranged atisine–hetidine, denudatine–denudatine, and heteratisine–denudatine.[11,13] Most of the isolated C_{20}-diterpenoid alkaloids are atisine-, hetisine-, and napelline-types.

2.3.1 Progress in the cytotoxicity research of C_{20}-diterpenoid alkaloids. Starting from Kashiwakura's work in 2007, numerous C_{20}-diterpenoid alkaloids have been tested for their cytotoxic activities against tumor cell lines.[18]

Atisine-type. In 2010, Gao's group reported a new diterpene alkaloid, delphatisine C (**62**) (Fig. 10), with significant cytotoxic activity (IC_{50} 2.36 μM) against the A549 cell line.[22] In the following year, a new diterpene alkaloid, honatisine (**63**) (Fig. 10), was isolated from *Delphinium honanense* and showed significant cytotoxic activity (IC_{50} 3.16 μM) against the MCF-7 cell line by SRB assay.[38]

Hetisine-type. Twenty-two C_{20}-diterpenoid alkaloids, including hetisine type and veatchine type, were tested for their effects on the growth of the A172, A549, HeLa and Raji tumor cell lines in 2009.[37] Only six hetisine-type derivatives, **64a–f** (Fig. 11), showed

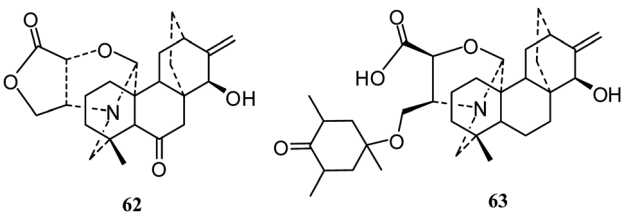
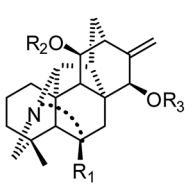
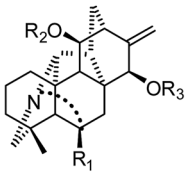


Fig. 10 The structures of atisine-type alkaloids **62** and **63**.



	R_1	R_2	R_3
64a	OH	*m*-CF_3-C_6H_5-CO	H
64b	OH	H	*m*-CF_3-C_6H_5-CO
64c	OH	As	H
64d	OH	Veratroyl	H
64e	OH	*p*-NO_2-C_6H_5-CO	H
64f	OH	Cinnamoyl	H



	R_1	R_2	R_3
65a	OAs	As	H
65b	OH	As	As
65c	OH	*p*-NO_2-C_6H_4-CO	H
65d	OH	*p*-NO_2-C_6H_4-CO	*p*-NO_2-C_6H_4-CO
65e	OH	Cinnamoyl	H
65f	OH	*m*-CF_3-C_6H_4-CO	H
65g	H	*p*-CF_3-C_6H_4-CO	H
65h	H	*m*-CF_3-C_6H_4-CO	H
65i	H	*p*-NO_2-C_6H_4-CO	*p*-NO_2-C_6H_4-CO

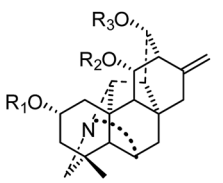
Fig. 11 The structures of hetisine-type alkaloids **64–65**.

significant suppressive effects; **64c** showed strong effects for the four cell lines, and **64d** showed the best effects against the Raji cell line. The IC_{50} values of **64a–f** against A549 cells were 4.4, 3.2, 1.7, 3.5, 3.5 and 5.1 μM, respectively.

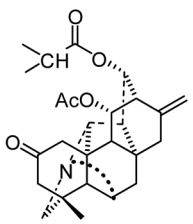
In 2011, thirty-nine hetisine type alkaloids were reported by Kashiwakura's group;[39,40] among these, eleven acyl derivatives (**64c–d**, **65a–i**) (Fig. 11) exhibited good cytotoxic activities, and 11,15-dianisoylpseudokobusine (**65b**) was found to be the most potent cytotoxic agent. Their IC_{50} values against A549 cells ranged from 1.72 to 5.44 μM.[39] 11-Anisoyl (As)-pseudokobusine (**64c**) and 11-m-trifluorometylbenzoyl (Mb)-pseudokobuisne (**65f**) also showed significant suppressive effects against Raji cells; their IC_{50} values were 2.2 μg ml^{-1} and 2.4 μg ml^{-1}, respectively.[40] Interestingly, no significant suppressive effects on the growth of human CD34 + hematopoietic stem/progenitor cells (HSPC) were observed for 11-Mb-pseudokobusine (**65f**), whereas 11-As-pseudokobusine (**64c**) showed significant suppressive effects on the growth of HSPC.

In 2014, Zhu and coworkers isolated five hetisine-type C_{20}-diterpenoid alkaloids, trichodelphinines A–E (**66a–e**), from *Delphinium trichophorum* Franch (Fig. 12). Their cytotoxic activities against A549 cancer cells were evaluated using the MTT method, and the IC_{50} values ranged from 12.03 to 52.79 μM.[41] The most active compounds, **66b** and **66e**, had lower IC_{50} values of 18.64 and 12.03 μM, respectively.

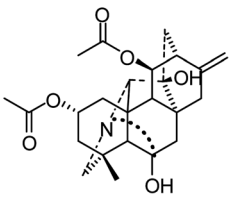
In 2015, the cytotoxic activities of seventy-two C_{20}-diterpenoid alkaloids were determined against the A549, DU145, KB, and KB-VIN cell lines (Fig. 12).[29] Fifty-two compounds were non-



	R_1	R_2	R_3
66a	H	Ac	$COCH(CH_3)_2$
66b	H	H	$COCH(CH_3)_2$
66c	H	Ac	$COCH(CH_3)CH_2CH_3$
66d	Ac	H	Ac



66f



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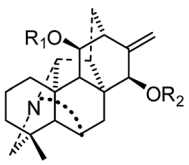
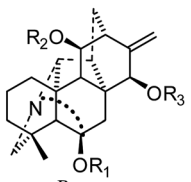


	R_1	R_2
67	Bz	Bz
68	As	As
69	*p*-NO_2-C_6H_4-CO	H
70	*p*-OCF_3-C_6H_4-CO	H
71	*p*-F-C_6H_4-CO	*p*-F-C_6H_4-CO
72	*p*-CF_3-C_6H_4-CH=CH-CO	*p*-CF_3-C_6H_4-CH=CH-CO



	R_1	R_2	R_3
73	Bz	Bz	H
74	H	Bz	Bz
75	Veratroyl	Veratroyl	H
76	Veratroyl	H	Veratroyl
77	H	*m*-NO_2-C_6H_4-CO	H
78	H	*p*-CF_3-C_6H_4-CO	H
79	H	Ph_3CH	H

Fig. 12 The structures of hetisine-type alkaloids **66–80**.

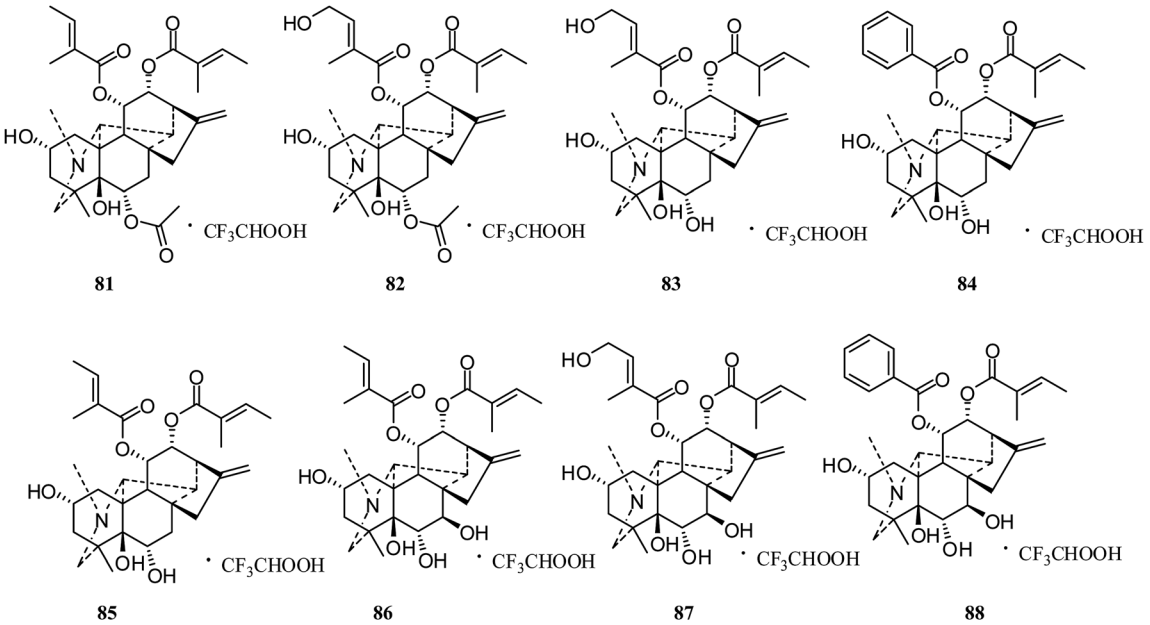


Fig. 13 The structures of anopterine-type alkaloid trifluoroacetate salts **81–88**.

toxic (IC_{50} > 20 μM), and nine derivatives (**61c–d**, **65g**, **69**, **70**, **73**, **75**, **76**, **78**) showed mild antiproliferative effects (IC_{50} = 10 to 20 μM). It was found that eleven derivatives (**64a**, **65c**, **65e**, **65i**, **67**, **68**, **71**, **72**, **74**, **77**, **79**), 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 (IC_{50} < 10 μM). Also, all of these were hetisine-type C_{20}-diterpenoid alkaloids with two different substitution patterns: C-11 and C-11, 15. The most potent compound, pseudokobusine 11,30-trifluoromethylbenzoate (**64a**), is a promising new lead that merits additional evaluation against multidrug-resistant tumors.[29]

The most recent report was published in 2017, where Shen *et al.* reported a new C_{20}-diterpenoid alkaloid, **80** (Fig. 12), that showed only low inhibition of the SK-OV-3 cancer cell line of 24% (IC_{50} 32.14 μM).[32]

Anopterine-type. In 2015, eight trifluoroacetate salts of anopterine-type diterpenoid alkaloids, 6α-acetoxyanopterine (**81**), 4′-hydroxy-6α-acetoxy-anopterine (**82**), 4′-hydroxyanopterine (**83**), 11α-benzoyl-anopterine (**84**), anopterine (**85**), 7β-hydroxyanopterine (**86**), 7β,4′-dihydroxyanopterine (**87**), and 7β-hydroxy-11α-benzoyl-anopterine (**88**) (Fig. 13), were reported by Davis's group; they appeared to be highly active toward prostate cancer cell lines, with IC_{50} values < 400 nM. Compounds **81**, **82**, and **88** were the most active, with IC_{50} values of 3.1, 10.9, and 6.7 nM against LNCaP cells, respectively.[42] Furthermore, live-cell imaging assays on **81–88** showed concentration- and time-dependent effects on the cell morphology and proliferation of LNCaP cells.

Delnudine-type. In 2014, Zhu and coworkers isolated one delnudine-type C_{20}-diterpenoid alkaloid, trichodelphinine F (**89**), from *Delphinium trichophorum* Franch (Fig. 14).[41] Its cytotoxic activity against A549 cancer cells was evaluated using the MTT method, with an IC_{50} value of 16.55 μM.

Napelline-type. In 2012, Gao *et al.* also reported two napelline-type C_{20}-diterpenoid alkaloids, songoramine (**90a**) and 15-cetylsongoramine (**90b**) (Fig. 14);[23] the latter showed lower cytotoxicity against HePG2 cells (IC_{50} 58.55 ± 9.17 μM).

Hetidine-type. In 2017, a new hetidine-type alkaloid, navicularine C (**91**), was isolated from the ground parts of *Aconitum naviculare*. However, it showed no cytotoxic activities against five tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480).[43]

2.3.2 The structure–cytotoxicity activity relationships of C_{20}-diterpenoid alkaloids. From 2008 to 2018, nearly 150 C_{20}-diterpenoid alkaloids have been studied for their cytotoxicity activities. In general, most of the active compounds were hetisine-type C_{20}-diterpenoid alkaloids; a very small number were anopterine type, napelline-type, or veatchine-type, while

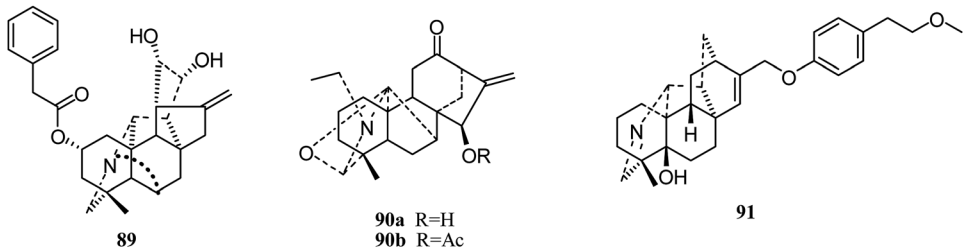


Fig. 14 The structures of other type C_{20}-alkaloids **89–91**.

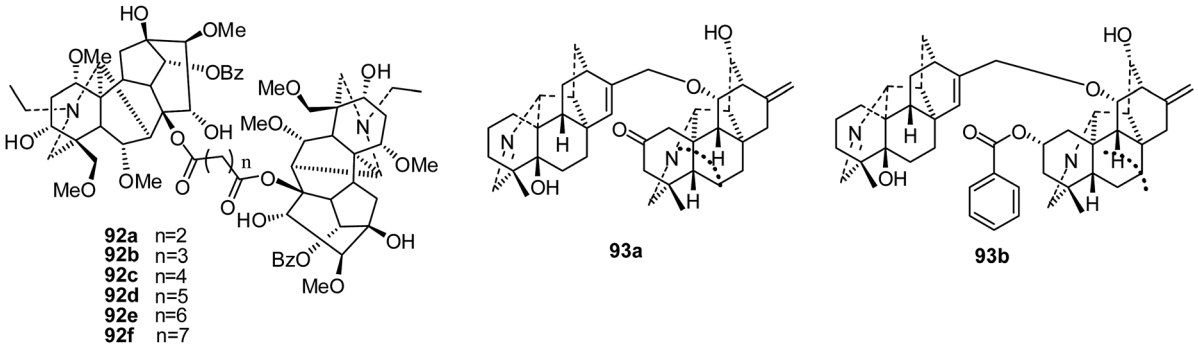


Fig. 15 The structures of bis-diterpenoid alkaloids 92–93.

only one delnudine-type, napelline type and hetidine type alkaloid have been studied, respectively.

In the cytotoxic effects of hetisine-type alkaloids, the hetisine-type structure, which is characteristic of C_{20}-diterpenoid alkaloids, plays a very important role in their pharmacological properties.[40] The results of the first cytotoxicity report of C_{20}-diterpenoid alkaloids suggested that the hydroxyl groups at C-6 and C-15 of hetisine-type compounds are necessary to their inhibitory effects. In particular, the C-11 residues are an important component for their anti-tumor properties and for their lower toxicity to hematopoiesis.[40] Replacement by an acyl group at both C-11 and C-15 resulted in enhanced activity of the parent alkaloids, and the presence of a hydroxy group at the C-6 position was required for the cytotoxic effects.[39] The identity of

Table 1 The plant sources of natural diterpenoids with cytotoxicity activities

Diterpenoid alkaloid type/class	Natural compounds	Plant source	Ref.
C_{18}	Lappaconitine (**16**)	*A. sinomontanum* Nakai	20
Lycoctonine-type C_{19}	Delpheline (**18**), delbrunine (**19**), delectinine (**20**),	*Delphinium chrysotrichum*	22
Lycoctonine-type C_{19}	*N*-Formyl-4,19-secopacinine (**45**), iminoisodelpheline (**46**), iminodelpheline (**47**)	*Delphinium elatum*	28
Aconitine-type C_{19}	Aconitine (**48**), chasmanine (**49**), crassicauline A (**50**), oxonitine (**51**), deoxyaconitine (**52**), hypaconitine (**53**), mesaconitine (**54**), senbusine A (**55**)	*Aconitum carmichaelii* Debx.	23
Aconitine-type C_{19}	Taipeinine A (**57a**), taipeinine B (**57b**), taipeinine C (**57c**)	*Aconitum taipeicum*	30
Aconitine-type C_{19}	15-Hydroxyldelphisine (**58**)	*Aconitum nagarum* var. *heterotrichum*	32
Aconitine-type C_{19}	Sinchiangensine A (**59**), lipodeoxyaconitine (**60**)	*Aconitum sinchiangense* W. T. Wang	33
Atisine-type C_{20}	Delphatisine C (**62**)	*Delphinium chrysotrichum*	22
Atisine-type C_{20}	Honatisine (**63**)	*Delphinium honanense*	38
Hetisine-type C_{20}	Trichodelphinine A (**66a**), trichodelphinine B (**66b**), trichodelphinine C (**66c**), trichodelphinine D (**66d**), trichodelphinine E (**66e**)	*Delphinium trichophorum* Franch	41
Hetisine-type C_{20}	14-Hydroxyl-2-acetoxy-spiradine C (**80**)	*Aconitum nagarum* var. *heterotrichum*	32
Anopterine-type C_{20}	6α-Acetoxyanopterine (**81**), 4′-hydroxy-6α-acetoxyanopterine (**82**), 4′-hydroxyanopterine (**83**), 11α-benzoylanopterine (**84**), anopterine (**85**), 7β-hydroxyanopterine (**86**), 7β,4′-dihydroxyanopterine (**87**), 7β-hydroxy-11α-benzoylanopterine (**88**)	*Anopterus macleayanus*	42
Delnudine-type C_{20}	Trichodelphinine F (**89**)	*Delphinium trichophorum* Franch	41
Napelline-type C_{20}	Songoramine (**90a**), 15-cetylsongoramine (**90b**)	*Aconitum carmichaelii* Debx.	23
Hetidine-type C_{20}	Navicularine C (**91**), navicularines A and B (**93a–b**)	*Aconitum naviculare*	43

the acyl group had varying effects. Simple benzoyl ester derivatives were generally less active. Cinnamoyl, *p*-nitrobenzoyl, *m*-trifluoromethylbenzoyl, and veratroyl substitutions were effective. Anisoate esters were generally found to be more potent than the other esters tested.[18,44] After further analysis of Lee's work,[29] it was further confirmed that all of the more active hetisine-type compounds had an ester or ether group on the C-11 hydroxyl and were either 11,15-diester analogs or 11-monoester/11,15-diester analogs.

For napelline-type, Gao and coworkers found that a compound with an additional acetyl group at C-15, 15-acetylsongoramine (**90b**), was more potent than songoramine (**90a**) against several human cancer cell lines; this shows that the ester groups play a considerably important role in the anticancer potency of these diterpenoid alkaloids.[23]

2.3.3 The cytotoxicity mechanism of C_{20}-diterpenoid alkaloids. When analyzing the cytotoxicity studies of C_{20}-diterpenoid alkaloids, only a few reports are found to focus on the mechanism of hetisine-type alkaloids.

According to the work of Kashiwakura's group, a mechanism study indicated that hetisine derivatives inhibit cell growth through G1 arrest. Furthermore, 11-Mb-pseudokobusine (**65f**) and **78** clearly inhibited the phosphorylation of extracellular signal-regulated kinase, induced enhanced phosphoinositide 3 kinase phosphorylation and led to subsequent accumulation of G1 and/or sub G1 phase in Raji cells.[29,39,40]

2.4 Bis-diterpenoid alkaloids

Only a few reports have focused on the cytotoxicity activities of bis-diterpenoid alkaloids.

In 2012, a series of bifunctional acyl compounds (**92a–f**) (Fig. 15) were also synthesized from the 8-*O*-azeloyl-14-benzoylaconine scaffold; their cytotoxicity activities were evaluated against a panel of human tumor cell lines, A-549, MCF-7 and HCT-15.[25] Surprisingly, three bis-[*O*-(14-benzoyl-aconine-8-yl)] esters (**92d–f**) bearing 7, 8 and 9 carbon atoms between the two aconitine moieties presented noticeable *in vitro* cytotoxic activities, with IC_{50} values ranging between 4 and 28 μM; this highlights the influence of the length of the alkyl linker. The most active compound, bis[*O*-(14-benzoylaconine-8-yl)]suberate (**92e**), was then evaluated *in vivo* in immunodeficient mice bearing human tumor xenografts originating from MCF-7 and HCT-15 cells. Antitumor activity was clearly detected at a dose of 10 mg kg^{-1}, which is far below the maximum tolerated dose (15 mg kg^{-1}).[25] Further studies on **92e** showed higher than average activities against leukemia and melanoma cell lines, especially SK-MEL-5 and SK-MEL-28, and against the COLO-205 and HT-29 (colorectal) and MDA-MB-468 (breast) cancer cell lines.[26] Also, it was implied that **92e** blocks the cell cycle at G2/M phase, displaying a mechanism of action related to that of nitrosoureas.[26]

In 2017, two new bisditerpenoid alkaloids, navicularines A and B (**93a–b**), were isolated from the ground parts of *Aconitum naviculare* (Fig. 15). Only navicularine B (**93b**) exhibited certain cytotoxic activities *in vitro*, with IC_{50} values of 13.50, 18.52, 17.22, 11.18, and 16.36 μM, respectively, against five cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480).[43]

3. Conclusions

Research on the cytotoxicity activities of more than 250 diterpenoid alkaloids has been reported in the past two decades. Some have shown effective anticancer properties in various cancer cell lines. These properties include inhibiting cell growth, inducing apoptosis, interfering with the cell cycle, and altering MDR.

In general, C_{20}-diterpenoid alkaloids have been studied more deeply than C_{19}-diterpenoid alkaloids. More derivatives of C_{20}-diterpenoid alkaloids have shown notable anticancer potential, such as **81–88**, with IC_{50} values < 400 nM. Meanwhile, some natural and synthesized C_{19}-diterpenoid alkaloids have shown anticancer effects, such as compounds **28**, **57a**, and **60** (with strong cytotoxicity activities comparable to those of adriamycin or cisplatin). Some also exert noteworthy anticancer effects in animal models.[25] Furthermore, many diterpenoid alkaloids tend to exhibit improved activity after simple structural modification;[27] many structures may affect the activity of a compound, such as the type and position of substituents and the linker-chain length.[45]

In brief, diterpenoid alkaloids have great potential as new drugs for treating cancer. However, the present research in this field is limited. Much additional research is required before these compounds can be applied as antitumor drugs (Table 1).

3.1 Selectivity between tumor cells and normal cells

As far as we know, most diterpenoid alkaloids with both active and toxic components are obtained from Ranunculaceae plants. Thus, the most important and urgent studies should focus on increasing the selectivity of tumor cells and normal cells to decrease their toxicity to the human body. However, work in this field is still lacking.

3.2 SAR study

The structure–cytotoxicity activity relationships (SAR) of diterpenoid alkaloids have not been deeply researched, particularly for C_{19}-diterpenoid alkaloids. In order to synthesize more potential active diterpenoid alkaloid derivatives, detailed SAR studies, such as quantitative structure–activity relationship (QSAR) studies, should be performed.

3.3 Further mechanism studies

The existing cytotoxicity mechanism studies for C_{19}- and C_{20}-diterpenoids are superficial; both mechanisms require full elucidation, which can also provide precise targets for further computer-aided design.

3.4 *In vitro* study to *in vivo* study

Finally and most importantly, the present reports are mostly restricted to *in vitro* tests. *In vivo* activity has been reported for only *in vivo* activities have been reported for only two compounds, **2** and **92e**. One compound, **92e**. Thus, a great step from *in vitro* to *in vivo* tests should be taken in the very near future to develop new preclinical antitumor diterpenoid alkaloid agents.

Conflicts of interest

There are no conflicts to declare.

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