Cytotoxic esterified diterpenoid alkaloid derivatives with increased selectivity against a drug-resistant cancer cell line

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Abstract

C-6 Esterifications of delpheline (**1**) were carried out to provide 20 new diterpenoid alkaloid derivatives (**4–22, 24**). Three natural alkaloids (**1–3**) and all synthesized compounds (**4–25**) were evaluated for cytotoxic activity against lung (A549), prostate (DU145), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KB-VIN) cancer cell lines and interestingly, showed an improved drug resistance profile compared to paclitaxel. Particularly, 6-(4-fluoro-3-methylbenzoyl)delpheline (**22**) displayed 2.6-fold greater potency against KB-VIN cells compared with the parental non-drug resistant KB cells. 6-Acylation of **1** appears to be critical for producing cytotoxic activity in this alkaloid class and a means to provide promising new leads for further development into antitumor agents.

Keywords

Delpheline; C_{19}-diterpenoid alkaloid; Cytotoxicity

Natural products have been the major sources of currently available anticancer drugs. According to a review of New Chemical Entities (NCE) from 1981 to 2006, approximately 73% of anticancer drugs were not purely synthetic compounds, with 47% being natural products, directly derived from natural products, or mimicking natural products in one form or another.[1] Diterpenoid alkaloids have been isolated from the genera *Aconitum, Consolida* and *Delphinium* of the Ranunculaceae family, and the genus *Spiraea* of the Rosaceae family. Diterpenoid alkaloids are classified according to their chemical structure as C_{18}-diterpenoid alkaloids, which have either a lappaconitine or a ranaconitine skeleton, C_{19}-diterpenoid alkaloids, which have either an aconitine, lycoctonine, lactone-type, pyro-type, rearranged-

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type, or 7,17-seco-type skeleton, and C_{20}-diterpenoid alkaloids, which have either an arcutine, atisine, denudatine, hetidine, hetisine, kusnezoline, napelline, racemulosine, tricalysiamide, or vakognavine skeleton.[2–4] The pharmacological properties of aconitine-type C_{19}-diterpenoid alkaloids, including aconitine, hypaconitine, jesaconitine, and mesaconitine, have been studied extensively and reviewed.[5,6] Two reports on the effects of C_{19}-diterpenoid alkaloids on cancer cells have appeared in recent years. 8-O-Azeloyl-14-benzoylaconine, an aconitine-type C_{19}-diterpenoid alkaloid, exhibited antiproliferative activity,[7] and the cytotoxic effects of various C_{19}-diterpenoid alkaloids against tumor cell lines were reported.[8] Our previous study demonstrated the effects of various naturally occurring and semi-synthetic C_{19}- and C_{20}-diterpenoid alkaloids on growth of the A172 human malignant glioma cell line.[9] Antitumor properties and radiation-sensitizing effects of various types of novel derivatives prepared from C_{19}- and C_{20}-diterpenoid alkaloids were also investigated.[10] Two novel hetisine-type C_{20}-diterpenoid derivatives showed significant suppressive effects against the Raji non-Hodgkin's lymphoma cell line.[11] In addition, the effects of various semi-synthetic novel hetisine-type C_{20}-diterpenoid alkaloids on the growth of the A549 human lung cancer cell line were examined and subsequent structure-activity relationships for the anti-proliferative effects against A549 cells were considered.[12] However, little information is available regarding the cytotoxic properties of lycoctonine-type C_{19}-diterpenoid alkaloids and their chemically transformed products. Herein, we report our study results on the unique cytotoxic activities of delpheline (**1**), a lycoctonine-type C_{19}-diterpenoid alkaloid, and its derivatives.

Delpheline (**1**), pacinine (**2**) and yunnadelphinine (**3**) were purified from the seeds of *Delphinium elatum* cv. Pacific Giant by a previously described procedure.[13,14] Delpheline (**1**) was reacted with various acyl chlorides at 80°C in pyridine to give C-6 substituted benzoyl and cinnamoyl derivatives (**4–22**). Delpheline (**1**) and 6-(3-trifluoromethylbenzoyl)delpheline (**12**) were hydrolyzed under acidic conditions to provide the 7,8-demethylene derivatives, 7,8-demethylenedelpheline (**23**)[15] and 7,8-demethylene-6-(3-trifluoromethylbenzoyl)delpheline (**24**), respectively. Oxidation of delpheline (**1**) with $KMnO_4$–acetone gave 19-oxodelpheline (**25**).

The three natural alkaloids (**1–3**) and all synthesized compounds (**4–25**) were evaluated for cytotoxic activity against four human tumor cell lines [lung carcinoma (A549), prostate carcinoma (DU145), nasopharyngeal (KB) and multi-drug resistant variant expressing P-glycoprotein (KB-VIN)]. Paclitaxel was used as a positive control (data shown in Table I).

Interestingly, the natural alkaloids (**1–3**) and synthetic analogs that did not contain a C-6 ester group (**23, 25**) were inactive ($EC_{50} > 20$ μM). Thus, acylation of the C-6 hydroxy group of **1** was necessary for cytotoxic activity. The methylenedioxy group of a lycoctonine-type C_{19}-diterpenoid alkaloid might also be necessary for an inhibitory effect against A549, DU145, KB and KB-VIN cell lines, as compound **24** lost activity against all cell lines compared with the related compound **12**.

Among the C-6 esterified compounds, **4** [6-(4-chlorobenzoyl)delpheline], **20** [6-(4-benzyloxybenzoyl)delpheline], and **21** [6-(3-fluoro-4-trifluoromethylbenzoyl)delpheline] exhibited the highest average potency over all cell lines (average EC_{50} 9.83, 9.57, and 9.41 μM, respectively). However, compound **16** [6-(3-trifluoromethylcinnamoyl)delpheline] showed significantly increased cytotoxic activity (EC_{50} 10.2 μM) against A549 cells compared with **4, 20**, and **21**, but was generally less potent against DU145, KB, and KB-VIN cells. Compounds **7, 11, 14**, and **17** were inactive against all four cancer cell lines, while **5, 6, 8–10, 13**, and **18** showed very limited potency.

The most striking observations from the data in Table 1 were the degree and relative ratio of KB/KB-VIN potency. Among the four cancer cell lines tested, the highest potency was found against the KB-VIN cell line by compounds **20–22** (EC$_{50}$ 4.22, 4.40, and 4.71 µM, respectively), followed by compounds **19, 15, 4, 16**, and **12** (EC$_{50}$ 6.50, 6.80, 8.27, 9.10, and 11.9 µM, respectively). Generally, all of the active compounds showed the highest potency against the KB-VIN cell line compared with the other three cancer cell lines tested. Moreover, compounds **4, 12, 20**, and **21** displayed similar potency against the KB and KB-VIN cell lines (ratio of EC$_{50}$ KB / EC$_{50}$ KB-VIN: 1.07, 1.25, 1.60, and 1.24, respectively). Even more notably, compounds **15, 19, 16**, and **22** showed over twofold selectivity between the two cell lines, with highest cytotoxic activity against the KB-VIN cell line (ratio of EC$_{50}$ KB / EC$_{50}$ KB-VIN: 2.15, 2.28, 2.31, and 2.57, respectively).

The identity of the substituent on the C-6 acyl group affected the cytotoxic potency. For instance, the compounds with the highest potency against the KB-VIN cell line contained chloro (**4**), fluoro (**15, 21, 22**), trifluoromethyl (**12, 16, 21**), ethoxy (**19**), or benzyloxy (**20**) substituents on the acyl group. Against the KB-VIN cell line, compounds **21** and **22** with both fluoro and trifluoromethyl/methyl groups were more potent than **12** with only a single trifluoromethyl group and even more potent than **5** with a single fluoro group. Similarly, compound **16** [6-(3-trifluoromethylcinnamoyl)delpheline] showed increased cytotoxic activity against most cell lines compared with the related fluorinated compounds **17** [6-(4-fluorocinnamoyl)delpheline] and **18** [6-(6-trifluoromethylnicotinoyl)delpheline]. Moreover, compounds with nitro, methoxy, phenyl, trifluoromethoxy, trifluoromethythio, and methyl carboxylate groups on a C-6 benzoate ester were generally less potent.

In conclusion, we have synthesized various C-6 acylated derivatives of **1**, a lycoctonine-type C$_{19}$-diterpenoid alkaloid. All derivatives were screened against four tumor cell lines, and the C-6 acylated was found to be critical for cytotoxic activity. Compounds **4, 12, 20**, and **21** displayed the greatest potency over all four tested cell lines. While these four compounds showed comparable potency against both KB and KB-VIN cancer cell lines, some compounds showed tumor-selective activity. Compounds **15, 16, 19**, and **22** exhibited greater inhibitory activity against drug-resistant KB-VIN cells (2.15–2.57 fold) than the parental KB cells. Continued studies are merited to further develop these promising new leads as antitumor agents, particularly with enhanced tumor selectivity.

Acknowledgments

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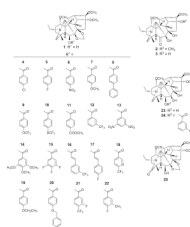
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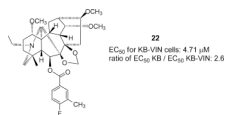
Figure 2.

Table 1

Cytotoxic activity data for diterpenoid alkaloid and derivatives [ED$_{50}$ (µM)] [a,b]

	A549	DU145	KB	KB-VIN
1	>20	>20	>20	>20
2	>20	>20	>20	>20
3	>20	>20	>20	>20
4	14.8 ± 3.76	7.40 ± 1.16	8.85 ± 2.02	8.27 ± 1.62
5	>20	15.6 ± 5.39	>20	15.0 ± 6.51
6	>20	17.2 ± 3.26	>20	17.7 ± 3.51
7	>20	>20	>20	>20
8	>20	17.1 ± 11.4	>20	17.4 ± 7.42
9	18.7 ± 6.57	20.3 ± 7.06	20.1 ± 7.63	18.9 ± 4.95
10	>20	16.6 ± 12.7	>20	17.9 ± 4.16
11	>20	>20	>20	>20
12	>20	12.6 ± 2.96	14.9 ± 4.93	11.9 ± 3.26
13	>20	>20	19.1 ± 4.78	20.3 ± 2.66
14	>20	>20	>20	>20
15	19.9 ± 10.1	16.9 ± 6.74	14.6 ± 7.05	6.80 ± 4.99
16	10.2 ± 2.62	15.1 ± 5.97	>20	9.10 ± 1.51
17	>20	>20	>20	>20
18	>20	>20	>20	18.7 ± 5.17
19	20.0 ± 0.89	15.6 ± 2.56	14.8 ± 3.27	6.50 ± 2.16
20	14.1 ± 2.93	13.2 ± 5.68	6.76 ± 1.74	4.22 ± 1.09
21	16.5 ± 2.24	11.3 ± 7.9	5.44 ± 1.75	4.40 ± 0.83
22	>20	19.8 ± 4.58	12.1 ± 7.83	4.71 ± 1.44
23	>20	>20	>20	>20
24	>20	>20	>20	>20
25	>20	>20	>20	>20
Paclitaxel	0.0064 ± 0.0013	0.0059 ± 0.0019	0.0060 ± 0.0008	0.76 ± 0.22

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

[b] Lung carcinoma (A549), prostate carcinoma (DU145), nasopharyngeal (KB) and multi-drug resistant variant expressing P-glycoprotein (KB-VIN).