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Multidrug-resistant cancer cell susceptibility to cytotoxic quassinoids, and cancer chemopreventive effects of quassinoids and canthin alkaloids

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Abstract—Twenty-three quassinoids (**1**–**23**), which were isolated previously from *Simaroubaceous* plants, were evaluated for cytotoxicity against three multidrug-resistant cancer cell lines, KB-VIN, KB-7d, and KB-CPT. Nine compounds (**2**–**7** and **9**–**11**) showed significant cytotoxicity in all three cell lines. Compounds **1**, **12**–**14**, **17**, and **20** demonstrated significant activity against the KB-7d and KB-CPT cell lines, and compounds **18**, **19**, and **23** revealed notable activity only against KB-7d cells. Structure–activity relationships were drawn based on these data. In addition, six quassinoid derivatives (**24**–**29**) and four canthin alkaloids (**30**–**33**), which were isolated from *Brucea antidysenterica*, were examined for their inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced Epstein–Barr virus early antigen (EBV-EA) activation as cancer chemopreventive agents. All of these compounds demonstrated significant inhibitory effects against EBV-EA activation.
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1. Introduction

Many quassinoids show various biological activities including antitumor,[1,2] antiAIDS,[3] antituberculosis,[4] insect antifeedant,[5] and inhibitory activities against Epstein–Barr virus early antigen (EBV-EA) activation.[6] Thus, in our continuing efforts to discover new antitumor agents from higher plants, we have been investigating quassinoids as well as canthin alkaloids of *Simaroubaceous* plants.[7–9] In an extension of this work, we report herein the cytotoxic activity of previously isolated quassinoids **1**–**23** (Figs. 1 and 2)[8] against multidrug-resistant cell lines (KB-VIN, KB-7d, and KB-CPT). Successful chemotherapeutic cancer treatment is severely hampered by multidrug resistance resulting from over-expression of the P-glycoprotein.[10–12]

In addition, considerable effort has been devoted to find effective cancer chemopreventive agents (antitumor promoters) from natural resources. Natural products including quassinoids,[13–16] flavonoids,[17,18] and triterpenoids[19] have been investigated for their inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced EBV-EA activation as cancer chemopreventive agents. In our previous paper, we reported the inhibitory effects of 45 natural quassinoids isolated from *Simaroubaceous* plants.[16] Based on the structure–activity relationship data, we postulated that reduction of the double bond in the A-ring of quassinoids, which have a 2-oxo-3-ene partial structure, led to increased inhibitory effects against EBV-EA activation. To assess this postulate, we tested six saturated quassinoids, **24**–**29** (Fig. 2), for their inhibitory activities.

Keywords: Multidrug resistance; Quassinoid; Canthin alkaloid; Cancer chemoprevention; Epstein–Barr virus activation; Structure–activity relationships.
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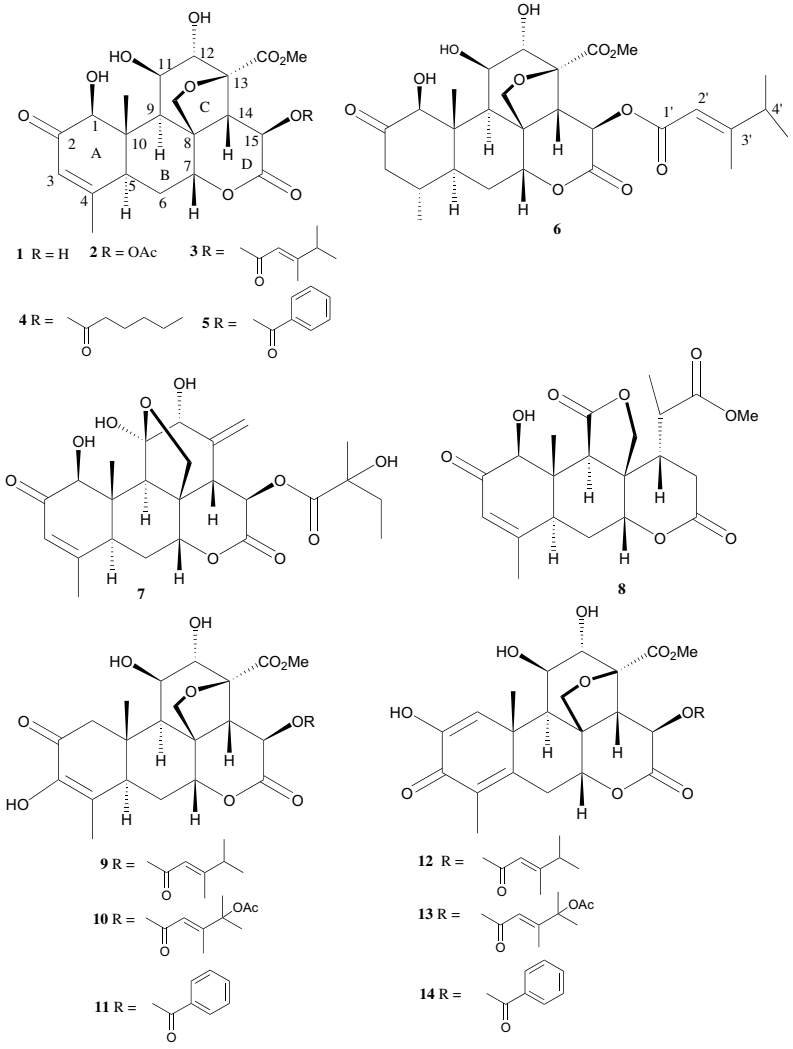

Figure 1.

Although canthin alkaloids isolated from *Simaroubaceous* plants and their derivatives have demonstrated cytotoxicity,[20,21] their inhibitory activities against EBV-EA activation by TPA in Raji cells have not been examined. Thus, we also report herein the results of the inhibitory effects of four canthin alkaloids **30**–**33** (Fig. 3) against EBV-EA activation.

2. Results and discussion

The previously isolated quassinoids were examined as inhibitors of human tumor cell line replication using the KB nasopharyngeal line and three multidrug resistant sublines, KB-VIN, KB-7d, and KB-CP. KB-VIN is a P-glycoprotein over-expressing derivative. KB-7d exhibits pleotrophic drug resistance to 4′-(9-acridinylamino)methanesulfon-maniside, doxorubicin, vincristine, and methotrexate, in part due to over-expression of the MRP transporter. KB-CPT is resistant to the growth inhibitory effect of camptothecin, but shows no cross-resistance to cisplatin and etoposide. Table 1 shows the resulting data against the KB cell line panel. Among the tested compounds, compounds **2**–**7** and **9**–**11** showed significant ($ED_{50} \leqslant 4\,\mu g/mL$) cytotoxic activity against the drug-resistant derivative KB-VIN with ED_{50} values ranging from 0.14 to 4.4 μg/mL. Compounds **1**–**7**, **9**–**14**, **17**–**20**, and **23** were active against KB-7d cells with ED_{50} values ranging from 0.0044 to 4.8 μg/mL. Compounds **1**–**7**, **9**–**12**, **17**, and **20** showed cytotoxic activity against KB-CPT cells with ED_{50} values ranging from 0.0062 to 2.0 μg/mL. Thus, among all tested compounds, **2**–**7** and **9**–**11** were significantly ($ED_{50} \leqslant 4\,\mu g/mL$) cytotoxic against all three drug-resistant variants (KB-VIN, KB-7d, and KB-CPT). As shown in Table 1, the cell lines demonstrated different susceptibility to the tested compounds. Among all compounds tested, compounds **6** and **9** exhibited the most significant activity against KB-7d and KB-CPT cells. Compound **9** also was the most potent compound toward the KB-VIN cell line. Compounds **1**, **13**, **14**, **17**, and **20** appeared to be substrates for P-glycoprotein, because the KB-VIN cell line exhibited significant cross-resistance to these compounds. When the cytotoxic activities of the tested compounds against the parental KB and KB-VIN cell lines were compared, all tested compounds were less active against the drug-resistant cell line. Also, none of the tested glycosides were active against KB-VIN cells.

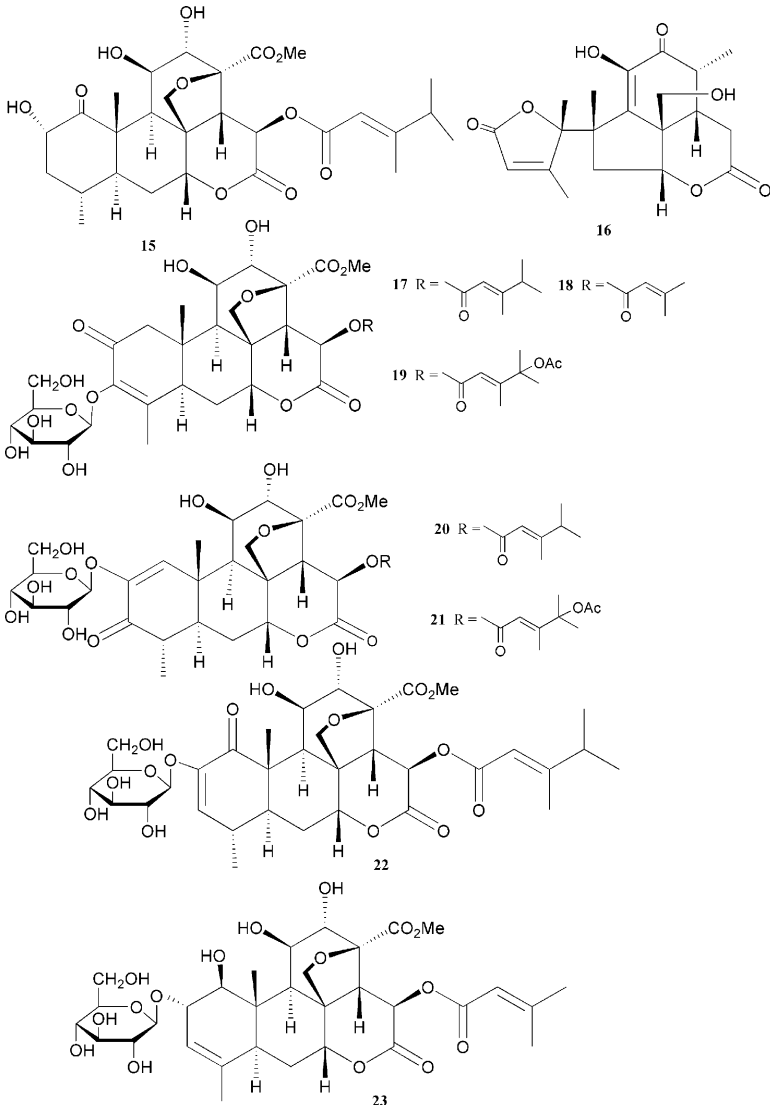

Figure 2.

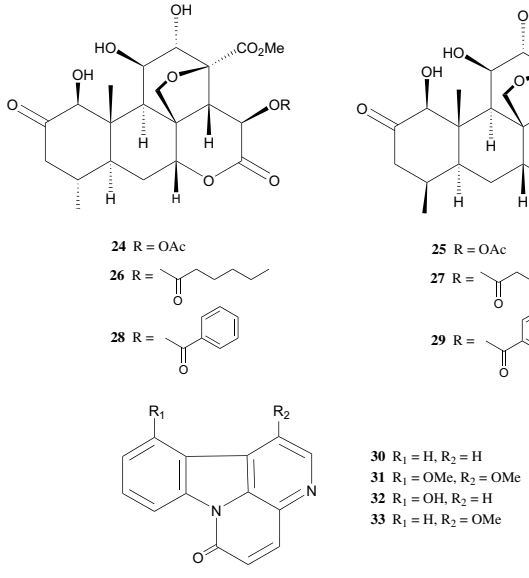

Figure 3.

Interestingly, although compounds **15**, **16**, **19**, and **21**–**23** were the least potent compounds in all four cell lines, they showed their greatest potency against the KB-7d cell line.

From a structure–activity perspective, the dehydro type moiety in the ring A resulted in reduced cytotoxic activity against all cell lines (compare **9**–**11** with **12**–**14**). Among the nine compounds effective against KB-VIN, seven compounds (**2**–**7** and **19**) contained the same 1-hydroxy-2-keto-3-ene partial structure in the A ring. A comparison of the structures and activities of compounds **20**, **22**, and **23**, which differ only in the partial structure of ring A, suggests that the presence of the ketone in ring A and the position of the conjugated enone system are important for the activity. Compound **8**, which has lost the C ring, also lost cytotoxic activity against all cell lines. On the other hand, compound **16**, which has a degraded A ring together with other structural changes, showed marginal activity only against KB-7d cells. The quassinoid glycosides **17**, **19**, **20**, and

Table 1. Cytotoxic activities of quassinoids **1**–**23**

Compd	IC_{50} (μM)/cell line			
	KB[a]	KB-VIN[a]	KB-7d[a]	KB-CPT[a]
1	0.79	20.5	0.77	2.0
2	0.0088	0.17	0.0079	0.015
3	0.0068	0.51	0.0060	0.0095
4	0.0077	1.0	0.0064	0.0080
5	0.0089	0.44	0.0081	0.010
6	0.0035	4.4	0.0050	0.0062
7	0.22	1.2	0.14	0.13
8	50.0	NA[b]	>50 (7)[c]	38.8
9	0.0052	0.14	0.0044	0.0066
10	0.0059	1.3	0.0062	0.0064
11	0.038	3.5	0.031	0.075
12	1.2	>50 (48)	1.1	1.7
13	0.033	6.6	0.024	0.034
14	0.17	7.7	0.14	0.18
15	5.2	NA	5.3	32.1
16	>50 (10)	NA	8.5	>50 (12)
17	1.1	34.8	0.47	1.3
18	1.3	>50 (8)	0.95	6.3
19	8.1	NA	4.2	9.1
20	1.5	45.1	0.65	1.9
21	6.8	NA	6.4	9.2
22	33.1	NA	5.8	36.0
23	27.7	NA	4.8	29.2
VP-16[d]	4.0	>50 (36)	9.4	4.5

[a] Cell line ED_{50} in μg/mL (replicates varied no more than 5%). For significant activity of pure compounds, an $ED_{50} < 4.0$ μg/mL is required.
[b] NA = Not active at the highest concentration indicated (<5% inhibition).
[c] If inhibition < 50% at 20 μg/mL, then the inhibition observed is given in parentheses.
[d] VP-16 = Etoposide.

21 were less active than corresponding aglycon quassinoids, **9** and **10**. Compounds **10**, **13**, **19**, and **21** were less active than **9**, **12**, **17**, and **20,** although their structures varied only in the presence of a C-4′ acetoxy group. The following activity comparisons revealed other SAR conclusions. The loss of the C-15 side chain in **2** reduced activity (compare **1** and **2**). Reduction of the C-3 double bond of **3** increased the activity against KB, KB-7d, and KB-CPT, but interestingly, decreased the activity against KB-VIN (compare **6** and **3)**. Interchanging the position of the C-1 hydroxyl and C-2 carbonyl groups markedly reduced activity (compare **6** and **15**). Based on the activity profiles shown in Table 1, compounds **2**–**7** and **9**–**11** may be useful leads for discovering drugs active against multidrug resistant tumors.

Six quassinoid derivatives (**24**–**29**) and each starting compound (**2**, **4**, and **5**) were examined for antitumor activity using a short term in vitro assay of TPA-induced EBV-EA activation in Raji cells (Epstein–Barr virus activation test). Table 2 shows the inhibitory effects and viabilities of the tested quassinoids. All assayed compounds demonstrated complete inhibitory activity at a concentration of 1000 mol ratio/TPA and maintained high viability of Raji cells (80%). These quassinoids also showed marked inhibitory activity at concentrations of 500, 100, and 10 mol ratio/TPA. Among all assayed quassinoids, compound **24** was the most potent compound and demonstrated significant activity with 100%, 100%, 96.5%, and 70% inhibition of activation at 1000, 500, 100, 10 mol ratio/TPA, respectively. The hydrogenated derivatives (**24**–**29**) showed slightly stronger inhibitory effect than the starting compounds (**2**, **4**, and **5**). Furthermore, quassinoids

Table 2. Inhibitory effects of quassinoids on TPA-induced EBV-EA activation

Compd	% EBV-EA positive cells[a]			
	Compound concentration (mol ratio/32 pmol TPA)[b]			
	1000	500	100	10
2	0 (80)[c]	2.5	7.5	32.5
24	0 (80)	0	7.5	30.0
25	0 (80)	0	7.5	32.5
4	0 (80)	7.5	25.0	37.5
26	0 (80)	7.5	22.5	32.5
27	0 (80)	7.5	22.5	35.0
5	0 (80)	2.5	22.5	47.5
28	0 (80)	0	20.0	45.0
29	0 (80)	0	20.0	45.0
30	0 (70)	26.7	84.9	100
31	0 (70)	21.5	80.8	100
32	0 (70)	0	36.3	82.2
33	0 (70)	19.3	85.0	100

[a] Positive control, 100%.
[b] Mol ratio/TPA (32 pmol = 20 ng/mL), 1000 mol ratio = 32 nmol; 500 mmol ratio = 16 nmol; 100 nmol ratio = 3.2 nmol; 10 nmol = 0.32 nmol.
[c] Values in parentheses are viability of Raji cells of the test compounds relative to the positive control (100%).

that have an α-oriented methyl group at C-4 exhibited stronger inhibitory effects than those with β-oriented groups.

Table 2 also shows the inhibitory effects of the four alkaloids on TPA-induced EBV-EA activation. Compounds **30**–**33** demonstrated 100% inhibition at 1000 mol ratio/TPA, and 100–73.3% inhibition at 500 mol ratio/TPA. These compounds maintained high viability of Raji cells at each assayed concentration (1000–10 mol ratio/TPA). Compound **32** was the most potent among the tested alkaloids, and it showed 63.7% inhibition activity at a concentration of 100 mol ratio/TPA. Compounds **31** and **33** were moderately active with values of 78.5% and 80.7% at 500 mol ratio/TPA, respectively. Compound **30** showed the weakest activity with a value of 73.3% at 500 mol ratio/TPA. From a structure–activity perspective, these results indicate that the hydroxy group is important for the activity (compare **32** with **30**), and addition of methoxy groups had less effect on activity (compare **31** and **33** with **30**).

3. Experimental

3.1. Test compounds

The isolation of quassinoids and canthin alkaloids from *Simaroubaceous* plants has been described in the literature.[1,2,5]

3.2. Cells

The human tumor cell line panel included KB (nasopharyngeal), KB-VIN (P-glycoprotein multidrug resistant), KB-7d (MRP multidrug resistant), and KB-CPT (MRP multidrug resistant) as described in detail elsewhere.[22,23]

3.3. Cytotoxicity assay

The assay was performed following the NCI's standard procedure using microtiter plate format and sulforhodamine-B (a cationic protein stain). Human tumor cell lines were continuously exposed to the test compounds for 3 days. Cells were cultured in RPMI-1640 growth medium, supplemented with 25 mM HEPES, 2% (w/v) sodium bicarbonate, 100 μg/mL kanamycin, and 10% (v/v) fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C. ED_{50}, the concentration that inhibited cell replication by 50% relative to control under the test conditions, was interpolated from graphed dose–response results (Graphpad Software, SanDiego, CA).

3.4. EBV-EA activation experiment

The inhibition of EBV-EA activation was assayed using methods reported in the literature.[24–26] The cells were incubated at 37 °C for 48 h in a medium containing butyric acid (4 nM), TPA (32 pM), and various amounts of test compounds. Smears were made from the cell suspensions and the EBV-EA-inducing cells were stained using an indirect immunofluorescence technique.

In each assay, at least 500 cells were counted, and the number of stained cells (positive cells) was recorded. Triplicate assays were performed for each data point. The EBV-EA inhibitory activity of the test compound was compared with that of the control experiment with butyric acid plus TPA. In control experiments, the EBV-EA inhibitory activities were generally around 40%, and these values were taken as a positive control. The viability of the cells was assayed by the trypan-blue staining method. For the determination of cytotoxicity, the cell viability was required to be more than 60%.

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