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13,28-Epoxy triterpenoid saponins from *Ardisia japonica* selectively inhibit proliferation of liver cancer cells without affecting normal liver cells

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ABSTRACT

Twenty 13,28-epoxy and related triterpenoid saponins from *Ardisia japonica* were evaluated for their anti-proliferative activity on human liver cancer cells and normal liver cells. Eight saponins selectively inhibited the growth of liver cancer Bel-7402 and HepG-2 cells without affecting the survival of normal liver HL-7702 cells. The structure–activity relationship analyses indicated that the 13,28-epoxy, 16α-hydroxy, and C-30 methyl moieties in the sapogenin parts and the glycosyl moiety consisting from tetra- to hepta-saccharide units are important for this activity. Among the active saponins, ardisianoside B (**2**) and 3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-16α-hydroxyoleanane (**3**) showed the most potent anti-proliferative activity against Bel-7402 cells in a dose- and time-dependent manner. The selective anti-proliferative activity is attributed to the different cellular responses (CDKs and cyclins levels, cell cycle arrest and apoptosis) between tumor and normal liver cells. Exposure to **2** and **3** selectively led to cell cycle arrest and apoptosis in Bel-7402 cells together with the increased pro-apoptotic caspase-8 and the decreased anti-apoptotic Cdc25A levels.

Liver cancer is the sixth leading type of cancers worldwide, and is also one of the four most prevalent malignant diseases in East Asia and sub-Africa.[1] To date, surgical resection is still the most effective treatment option for liver cancer, but is available only for a small portion of patients with a high recurrence rate. Chemotherapy is also commonly used for the liver cancer patients who are not feasible for surgery, or used as an adjuvant treatment in addition to surgery. However, severe toxic side effects, low tumor-selectivity, and highly metastatic and chemo-resistant nature of liver cancer greatly hampered the effectiveness of chemotherapy.[2] Thus, development of more effective drugs possessing high tumor-selective pro-apoptotic activity is critical.

The triterpenoid saponins with a 13,28-epoxy moiety in the sapogenin structure are characteristic constituents in the genera of *Ardisia*, *Maesa*, and *Myrsine* from the plant family of Myrsinaceae, as well as in the genera of *Cyclamen*, *Lysimachia*, and *Primula* from the family of Primulaceae, and have been reported to exhibit a large range of bioactivities, such as antileishmanial and antiviral activities.[3] Numerous compounds belonging to this type of triterpenoid saponins have also been reported to cause potent cytotoxicity against a variety of human cancer cell lines through various mechanisms, such as cell cycle arrest and apoptosis induction and microtubule disassembly, demonstrating the potential anti-cancer property of these compounds.[4–8]

As part of our ongoing study on discovery of new anti-cancer compounds from natural sources, we report here the evaluation of 13,28-epoxy triterpenoid saponins from a traditional Chinese medicinal plant *Ardisia japonica* for their in vitro tumor-selective anti-proliferative activities, and the underlying molecular mechanisms involved was also elucidated.

Triterpenoid saponins (**1**–**20**) used in this study were isolated from *A. japonica* as in a previous report.[4] The purity of the tested compounds was >98% as identified by HPLC and NMR. According to the view that an ideal anti-cancer drug should selectively kill cancer cells without affecting normal cells, MTT assay was conducted to assess the selectivity of their anti-proliferative activities in both Bel-7402 and HepG-2 liver cancer cell lines and HL-7702 normal liver cells.[9] While the positive control epirubicin markedly inhibited the growth of both tumoral and normal liver cells, 8 out of 20 triterpenoids displayed a selective growth inhibitory activity on Bel-7402 and HepG-2 cells (IC_{50} 0.92–31.21 μM) without affecting the survival of HL-7702 cells (Fig. 1, Table 1).

The structure–activity relationship was concluded by comparison of the structure and anti-proliferative activities between these compounds. Variation of the structures including both sapogenin and C-3 glycosyl moieties affects the activities. Structural related saponins (**18**, **19**, **20**) with the same C-3 glycosyl moiety as saponin **3** have shown no activity, suggesting that the 13,28-epoxy moiety

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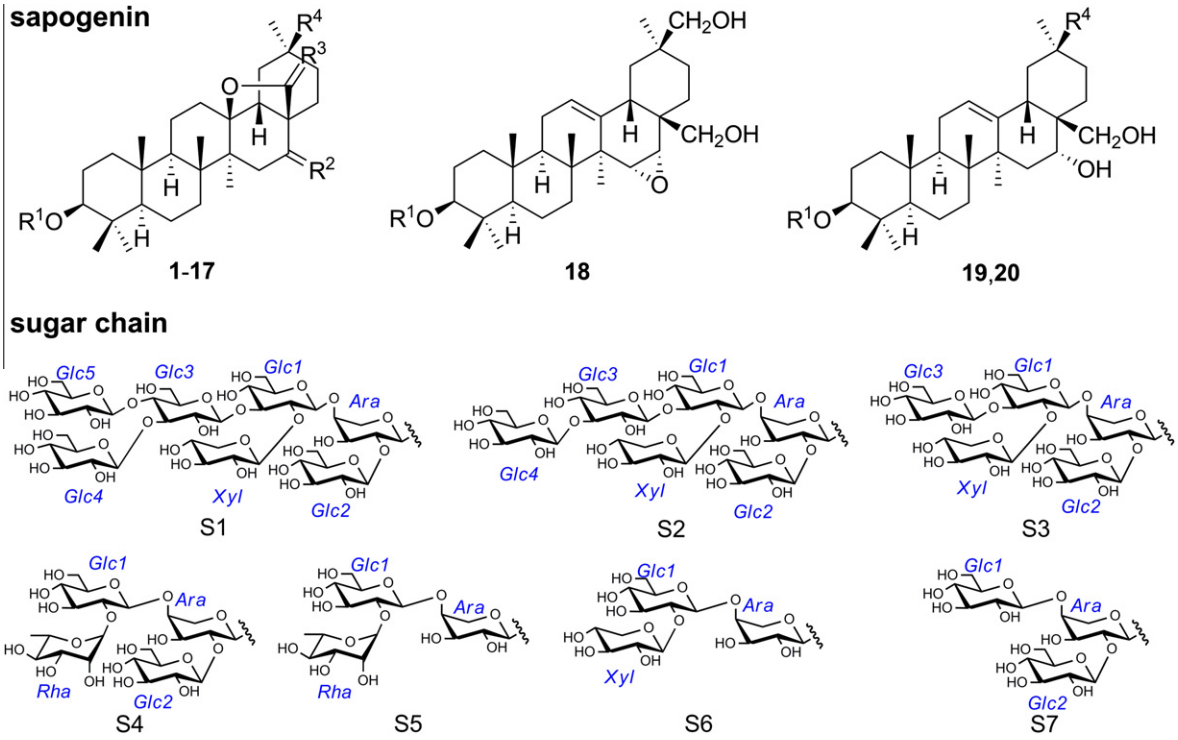

Figure 1. Structures and anti-proliferative activities of compounds **1**–**20**.

Table 1
Structures and anti-proliferative activities of compounds **1**–**20** against tumoral and non-tumoral human liver cells

Compounds	R^1	R^2	R^3	R^4	IC_{50}[a]		
					Bel-7402	HepG-2	HL-7702
1	S1	α-OH	H_2	CH_3	1.37 ± 0.10	3.83 ± 0.15	NA[b]
2	S2	α-OH	H_2	CH_3	0.92 ± 0.18	2.98 ± 0.84	NA
3	S4	α-OH	H_2	CH_3	1.18 ± 0.21	3.17 ± 0.55	NA
4	S5	α-OH	H_2	CH_3	4.14 ± 0.25	13.62 ± 1.03	NA
5	S6	α-OH	H_2	CH_3	14.78 ± 2.17	31.21 ± 1.55	NA
6	S7	α-OH	H_2	CH_3	4.18 ± 0.33	14.43 ± 0.62	NA
7	S4	α-OH	H_2	CH_2OH	NA	NA	NA
8	S3	α-OH	H_2	CHO	3.85 ± 0.07	12.19 ± 0.22	NA
9	S4	α-OH	H_2	CHO	4.46 ± 1.18	20.96 ± 0.36	NA
10	S5	α-OH	H_2	CHO	NA	NA	NA
11	S6	α-OH	H_2	CHO	4.05 ± 0.26	NA	NA
12	S5	=O	H_2	CHO	NA	NA	NA
13	S4	=O	H_2	COOH	NA	NA	NA
14	S4	α-OH	H_2	OH	NA	NA	NA
15	S1	α-OH	=O	CH_3	NA	NA	NA
16	S4	α-OH	=O	CH_2OH	NA	NA	NA
17	S5	α-OH	H_2	$CH(OCH_3)_2$	NA	NA	NA
18	S4				NA	NA	NA
19	S4			CH_2OH	NA	NA	NA
20	S4			COOH	NA	NA	NA
Epirubicin[c]					1.23 ± 0.36	2.23 ± 0.81	1.74 ± 0.17

[a] Values are means ± SD, $n = 3$, IC_{50} in mM.
[b] IC_{50} >100 mM.
[c] Positive control.

in the sapogenin part is necessary to exhibiting the cytotoxic activity. In saponins (**13**, **15**, **16**), existence of C-28 lactone moiety or C-16 ketone moiety also eliminate the anti-proliferative activity. Function moieties at C-30 are important for anti-proliferative activity. The activities of saponins **3** > **9** > **7**, **13**, **14**, suggested that C-30 in the sapogenin part are preferable to be methyl moiety rather than methyl aldehyde moiety, and not to be carbonyl or hydroxymethyl moiety or in nor type (30-hydroxy moiety). As for the C-3 glucosyl moiety, a sugar chain consisting from tetra- to hepta-saccharide units are seems preferable to exhibit the activity, and the tetrasaccharide units of β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl or α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl are the core structures. In saponins (**4**, **5**, **6**, **10**, **11**) with a C-3 glycosyl moiety to be tri-saccharide units, absence of either the