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Anticancer effects of a novel class rosin-derivatives with different mechanisms

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ABSTRACT

In this Letter, the anticancer activity of novel rosin-derivatives introducing indicated side chains at position C18 of dehydroabietic acid (DHAA) was reported. Gratifyingly, all of these derivatives showed significantly cytotoxicity toward diverse human carcinoma cell lines. We found the compound **4** could induce cell membrane damage at high concentration as well as cell apoptosis at low concentration. However, compound **5**, attachment of quinidine to dehydroabietic acid via thiourea bond, only induced apoptotic cell death. In addition, all these active compounds induced apoptosis mainly through mitochondrial-dependent pathway. Interestingly, compound **5** exhibited the highest anticancer activity and little toxicity to normal cells compared with the other derivatives. Therefore, **5** merits further investigation as a potential agent for future anticancer treatment.

Cancer still remains a threat to men's health, representing the second leading of death worldwide.[1] There has been increased interest in the search of chemotherapeutic drugs from natural sources, and many natural or natural based antitumor drugs such as taxol and vinblastine were found and clinically used in recent years.[2,3] Dehydroabietic acid (DHAA) (Fig. 1, compound **1**), a natural occurring diterpene rosin acid, is extracted from *Pinus* rosin or commercial rosin and its derivatives have attracted great interest for their broad-spectrum biological activities including antiulcer, antimicrobial, anxiolytic, antiviral and antitumor activities.[4–8]

Quinidine (Fig. 1, compound **2**), a natural alkaloid, wide range of biological activities made it used as antimalarial and antiarrhythmic agents.[9,10] In addition, quinidine and its derivates were reported to have properties of enhancing the inhibitory activity of anticancer drugs by suppressing the activities of P-glycoprotein and Cytochrome P450, which act as efflux pump and drug-metabolizing enzyme for various anticancer drugs.[11,12]

Previously, we have synthesized a novel class of rosin-derived catalysts, which have successfully been applied to several asymmetric transformation processes.[13–17] These effective derivatives were synthesized by attaching different functional groups to the DHAA at the C18 position via a thiourea bond, which has been extensively used in the conjugated drugs.[18,19] In light of these research results, studies were designed to elucidate the effects of these rosin-derivatives (**4**–**8**) and native compounds (**1**–**3**) on the proliferation of various cancer cell lines (Fig. 1). Furthermore, we also studied the killing mechanism of these derivatives by distinct methods.

The anticancer activity of these compounds was assessed in two bladder carcinoma cell lines (EJ and 5637), one prostate carcinoma cell line (PC-3). (Table 1), one cervical carcinoma cell line (Hela) and one human T-cell leukemia cell line (Jurkat) with 72 h treatment (the IC_{50} in Hela and Jurkat was shown in Supplementary data, Table S1). Our results showed that all of these derivatives (compounds **4**–**8**) exhibited high cytotoxicity to cancer cells. However, DHAA, quinindine and dehydroabietic amine (compounds **1**–**3**) displayed lower anticancer activity compared with theses derivatives. Compound **5**, attachment of quinidine to DHAA via thiourea bond, showed the highest potent anticancer activity among these tested compounds. In addition, the cytotoxicity of the compounds **2**, **3**, **4** and **5** was further measured in bladder carcinoma cell line (EJ) and cervical cancer cell line (Hela) (see Supplementary data, Fig. S1) for the indicated term of incubation. As shown in Figure 2, after 1 h of treatment with compounds **3** and **4** at high concentrations (10 and 20 μM), cell death could be detected significantly. However, compound **2** and **5** had almost no effect on the proliferation of tested cancer line within 1 h incubation even at the highest concentration (20 μM). Interestingly, the incubation time of these four compounds exceeding 24 h, compound **5** exhibited the best cycotoxicity to EJ cells.

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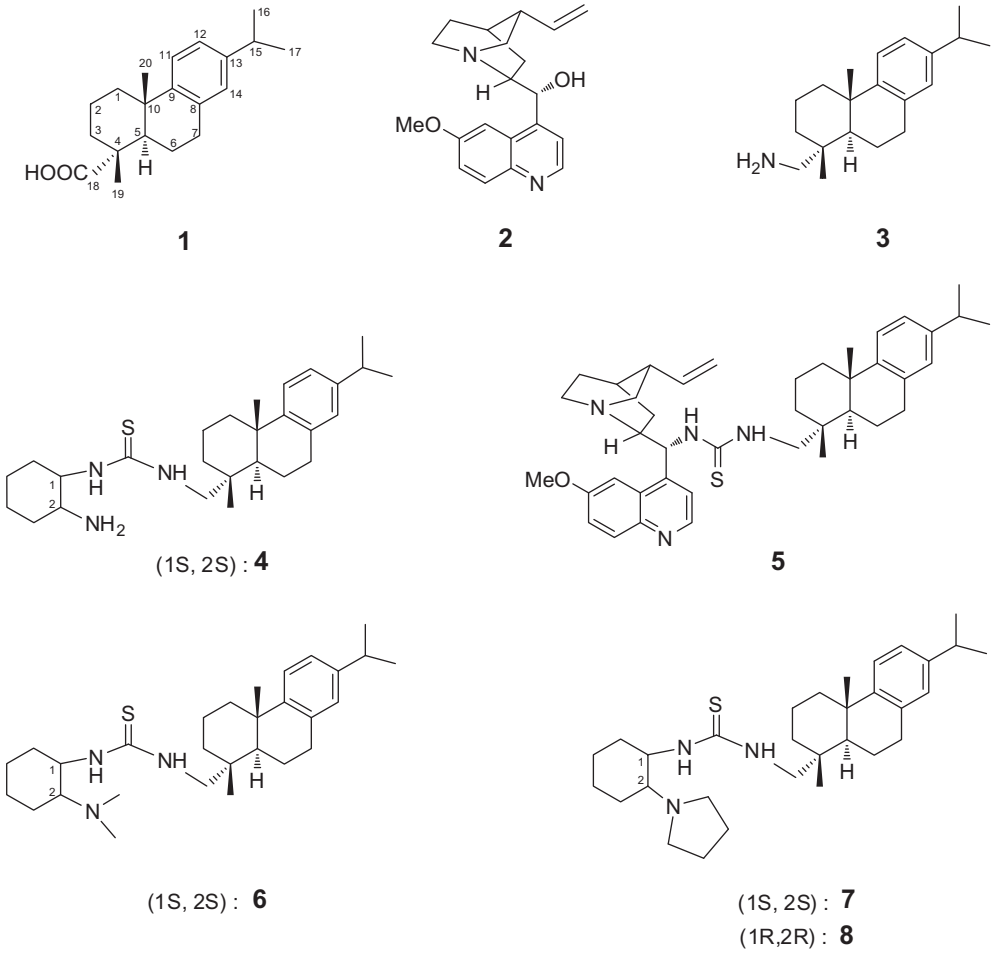

Figure 1. Chemical structures of rosin-derivatives and native compounds.

Table 1
In vitro anticancer activities of compounds 1–8

Compd	IC_{50}[a] (μM)		
	5637	PC-3	EJ
1	27.59 ± 1.13	28.10±0.83	25.12 ± 1.70
2	>50	>50	>50
3	8.70 ± 0.25	7.88 ± 0.43	4.82 ± 0.22
4	3.14 ± 0.13	3.52 ± 0.25	3.05 ± 0.18
5	2.64 ± 0.10	2.98 ± 0.13	2.07 ± 0.17
6	4.62 ± 0.08	5.53 ± 0.22	4.19 ± 0.05
7	5.18 ± 0.10	6.57 ± 0.19	4.29 ± 0.10
8	3.59 ± 0.14	4.70 ± 0.20	3.44 ± 0.18

[a] IC_{50} (μM) is 50% inhibitory concentration, and values are means ± SE. of three experiments each done in duplicate.

It was reported DHAA could disrupt the red cell membranes, leading to hemolysis.[20] Therefore, we used hemolysis assay to evaluate the toxicity of active compounds **2**, **3**, **4** and **5** to normal cells. Our results indicated that treatment of mice erythrocytes with compounds **3** and **4** could induce apparent erythrocytes lysis (17.88%, 74.44%, respectively) at 100 μM (Fig. 3). In contrast, compounds **2** and **5** showed no hemolytic activity even at high concentrations. Taken together, the attachment of quinidine could significantly reduce the toxicity of DHAA to normal cells compared with other functional groups.

Because compounds **3** and **4** rapidly induced cell death at high concentrations, so we presumed that these active compounds may kill cells by disrupting membrane integrity just like antimicrobial peptides.[21] PI, one cationic dye, is excluded by the membranes of viable cells but can pass through the damaged plasma membranes.[22] To verify the membrane disruption activity of **3**, **4** and **5**, we studied the change of cell membrane integrity by detecting the uptake of PI in EJ cells after compounds treatment. As shown in Figure 4A, the membranes of control and cells treated with **5** were integral, exhibited normal and smooth morphology, and negative to PI. However, cells incubated with **3** and **4** showed disrupted membranes, and most of them were stained by PI. In addition, EJ cells exposure to compounds **6**–**8** stained rapidly and intensely with PI (PI staining for compounds **6**–**8** was shown in Supplementary data, Fig. S2). Furthermore, scanning electron microscopy (SEM) was used to examine the subtle morphologic changes on the EJ cell membrane after compounds treatment. Similar to the untreated EJ cells, most EJ cells treated with compound **5** showed plenty of microvilli and adherent smooth surface. Following the treatment with compound **4**, EJ cells were heavily disrupted and cell membranes were characterized with significant pore formation and loss of microvilli (Fig. 4B). The result derived from SEM was consistent to that derived from PI uptake assay, confirming that **3**, **4** kill tumor cells via the membrane disruption mechanism, just like many naturally occurring antimicrobial peptides but distinct from **5**. Although **5** and **3**, **4** were all derived from DHAA, attaching different functional groups at the C18 position resulted different anticancer mechanisms.

Compounds **3** and **4** not only induced cell membrane disruption at high concentration but also killed cell at low concentration when incubation term exceeding 24 h, suggesting an apoptosis