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Dispirocyclopropyldehydrocostus lactone selectively inhibits acute myelogenous leukemia cells

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ABSTRACT

Acute myeloid leukemia (AML) is a refractory disease, and the majority of AML patients died from relapse and multidrug resistance. More and more studies demonstrate that AML stem cells play key role in multidrug resistance of AML. Here, we report a derivative of dehydrocostus lactone, that is, dispirocyclopropyldehydrocostus lactone (DDL), showed preferable cytotoxicity against a series of leukemia cell lines and AML stem cells from clinical samples of AML patient. Meanwhile, DDL demonstrated no significant toxicity to normal hematopoietic cells. Therefore, the prodrug of DDL, DMADDL, was evaluated for its in vivo anti-AML activity. The result revealed that DMADDL could inhibit the tumor growth in SCID mice tumorigenicity assay. Further study suggested that DDL induced apoptosis mainly through the up-regulation of apoptosis related protein Bax, followed by the cleavage of caspase-3, caspase-9, and PARP.

Acute myeloid leukemia (AML) is a heterogeneous group of leukemia cells that results from hematopoietic stem cell disorder through multiple gene mutation and chromosomal rearrangements.[1,2] As one of the most common lethal leukemia, AML typically leads to death with weeks to months in clinical presentation. It is usually treated with chemotherapy or stem cell transplantation. The chemotherapy included cytarabine, daunorubicin, elacytarabine,[3] CPX-351,[4,5] clofarabine,[6] sapacitabine,[7] vosaroxin.[8] Remission rates in adult patients with AML could effectively controlled, however, it is hard for elder patients to achieve a complete remission and most AML patients will relapse within two years.[9–11] Moreover, patients over age 60 have a significantly poor prognosis; the 5-year survival is 10% or less.[12]

Clinical trials conducted that traditional treatment was demonstrated to induce remission in approximately 60% of AML patients, which is associated with clinical drug resistance.[13] Moreover, cytarabine-based and adriamycin (ADR)-based drug resistance is increased. The multidrug resistance-associated ATP-binding cassette proteins ABCB1 (MDR1, P-glycoprotein), ABCG2 (breast cancer resistance protein) and ABCC1 (multidrug resistance-associated protein 1) is often overexpressed in AML drug resistance specimens.[14] The multidrug resistance inhibitors (valspodar, zosuquidar trihydrochloride) have been verified in relapsed AML treatment.[15] Although the multidrug resistance happened frequently, contemporary treatment regimens are still based nucleoside analogs, anthracyclines, and intensive post remission therapy.[16] More and more studies demonstrate that AML stem cells are the root of relapse and multidrug resistance of AML.[17–20] However, there are barely few compounds that can selectively inhibit AML stem cells.[21] Therefore, novel therapeutic agents with selective activity against AML stem cells are urgently needed.

Dehydrocostus lactone (Fig. 1), a natural guaianolide sesquiterpene lactone,[22] was isolated from the roots of *Saussurea lappa*, a traditional Chinese medicinal herb.[23] Recently, we found that dehydrocostus lactone exhibited inhibitory activity against human AML progenitor cells.[24] However, during the process of separating dehydrocostus lactone from the roots of *Saussurea lappa* in our laboratory, we found that dehydrocostus lactone was prone to polymerize. Corona et al also reported that dehydrocostus lactone eased to undergo fast polymerization.[25] We supposed that the two unconjugated exo-double bonds of dehydrocostus lactone might be responsible for its polymerization. Accordingly, we synthesized a dispirocyclopropyl derivative of dehydrocostus lactone, that is, dispirocyclopropyldehydrocostus lactone (DDL).[26] Herein, we demonstrate that DDL showed preferable cytotoxicity against a series of leukemia cell lines and AML stem cells from clinical samples of AML patient. Meanwhile, DDL indicated no significant cytotoxicity against normal hematopoietic cells. The prodrug of DDL, DMADDL, could inhibit the tumor growth in SCID mice

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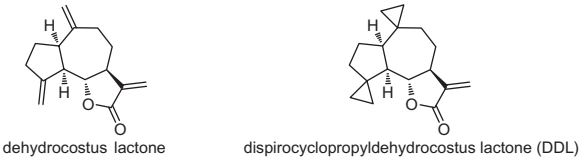
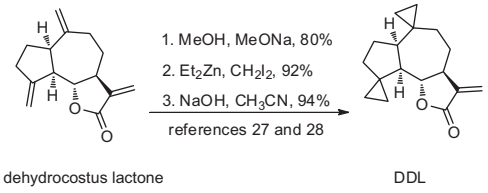


Figure 1. The structures of dehydrocostus lactone and its derivative dispirocyclopropyldehydrocostus lactone (DDL).



Scheme 1. Synthesis of DDL.

tumorigenicity assay. In addition, DDL induced apoptosis mainly through the up-regulation of apoptosis related protein Bax, followed by the cleavage of caspase-3, caspase-9 and PARP.

The synthesis DDL was followed our reported procedure (Scheme 1).[26,27] DDL was evaluated as a cytotoxic agent to different cancer cell lines in vitro. The cell lines used for this study included chronic myeloid leukemia (CML) cell lines K562 and K562/A02, and AML cell lines HL60, HL60/ADR and KG1a, and adriamycin (ADR, a clinically popular anti-AML agent) was used as positive control. The results are summarized in Figure 2 and Table 1. After incubated for 72 h all cell lines were sensitive to DDL with IC_{50} values ranging from 5.7 μM to 7.7 μM (Table 1). These results suggest that bioisosteric replacement of the two unconjugated double bonds by cyclopropyl moieties maintains the anti-cancer activities. For DDL, the activity against drug-resistant cell line HL-60/ADR was comparable to that against sensitive cell line HL60 (IC_{50} = 6.7 μM vs IC_{50} = 5.7 μM), while ADR exhibited a potency difference of more than 300 times against HL60/ADR (IC_{50} = 4.89 μM) and HL60 (IC_{50} = 0.016 μM) (Table1). Furthermore, DDL exhibited little cytotoxicity to normal hematopoietic cells which were obtained from umbilical cord blood (IC_{50} >50 μM, Fig. 2F).

Previous study suggested that KG1a cells exhibited highest p-glycoprotein-mediated drug efflux capacity and a high level of DNR resistance,[28] it is a type of short-term CD34+ hematopoietic progenitor cell line, and it contained leukemia stem-like cells CD34+CD38−,[29] in some cases, the leukemic stem-like cells occupy about 50% of the KG1a cell line.[30] Moreover, the leukemic stem-like cells are highly mutidrug-resistant and NK cell-resistant.[31] It is noteworthy that DDL exhibited high cell-growth inhibition (IC_{50} = 7.6 μM) to KG1a leukemia cell line, which is comparable to that against HL60 (IC_{50} = 5.7 μM) and HL60/ADR (IC_{50} = 6.7 μM). However, ADR showed a potency reduction of 50 times against KG-1a (IC_{50} = 0.82 μM vs IC_{50} = 0.016 μM against HL60).

DDL was further screened in an assay against leukemia cells isolated from blood samples of AML patient (Table 2). The cell viability was determined by annexin labeling after 18 h of treatment. At a concentration of 10 μM, the cell viability of CD34+ cells after treatment with DDL was 1.9%, while the cell viability of CD34+ cells after treatment with ADR was 49.1%. Moreover, after treatment with DDL, the cell viability of CD34+CD38− cells was 0.46%. These data indicated that DDL selectively eradicate AML progenitor cells and stem cells.

In order to investigated whether DDL showed cytotoxicity to KG1a cells through the induction of apoptosis, the percentage of apoptosis was analyzed by flow cytometric assay. After the treatment of DDL, the cells were collected and stained with Annexin V-FITC and PI. The percentage of apoptosis were 4.9%, 10.1%,

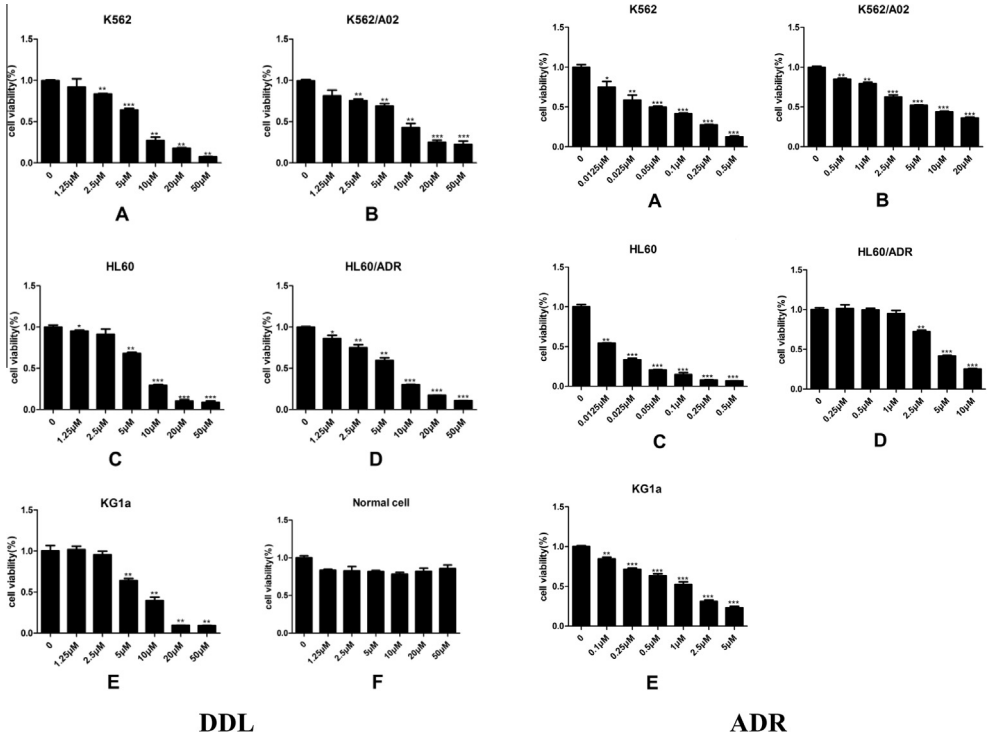


Figure 2. Acitivities of DDL and ADR against a series of tumor cell lines after incubating for 72 h. (A) DDL and ADR inhibited K562 cell viability; (B) DDL and ADR inhibited K562/A02 cell viability; (C) DDL and ADR inhibited HL60 cell viability; (D) DDL and ADR inhibited HL60/ADR cell viability; (E) DDL and ADR inhibited KG1a cell viability; (F) DDL showed little effect on normal hematopoietic cells which were isolated from umbilical cord blood after incubating for 24 h.