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Design and synthesis of novel nitrogen mustard-evodiamine hybrids with selective antiproliferative activity

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ABSTRACT

A series of novel nitrogen mustard-evodiamine hybrids were synthesized and evaluated for their antiproliferative properties. The antiproliferative activities of **10a–d**, **11a–d**, and **12a–d** against four different kinds of human cancer cell lines (PC-3, HepG2, THP-1 and HL-60) and human normal peripheral blood mononuclear cells (PBMC) were determined. The results showed that all the target hybrid compounds exhibited antiproliferative activities against tested human tumor cell lines to some extent and no antiproliferative activities (>200 μM) against human normal PBMC cells. The antiproliferative selectivity between tumorous and normal cells was very useful for further antitumor drug development. Among the target compounds, **12c** showed the strongest cytotoxicity against two tumor cell lines (THP-1 and HL-60) with IC_{50} values of 4.05 μM and 0.50 μM, respectively, and selected for further mechanism study in HL-60 cells. The results showed that **12c** could induce HL-60 cells apoptosis and arrest at G_2 phase at low submicromolar concentrations via mitochondria-related pathways.

Alkaloids have long played important roles in cancer chemotherapy.[1] Evodiamine (**1**, Scheme 1) is a quinolone alkaloid isolated from the fruits of traditional Chinese herb *Evodiae fructus*, which is widely used for the treatment of diverse human disorders.[2] Previous studies showed that evodiamine possessed extensive biological activities, such as antitumor,[3–5] anti-Alzheimer's disease,[6] anti-inflammatory[7,8] and so on.[9,10] Recent studies have reported that evodiamine exhibited cytotoxicity against a wide variety of tumor cells *in vitro* and antitumor activities *in vivo* by mainly inducing apoptosis or cell cycle arrest, and inhibiting topoisomerase I and II, angiogenesis, invasion, and metastasis.[11] In addition, many researchers tried to elucidate the mechanisms of its antitumor effects. For instance, evodiamine could block STAT3 signaling pathway by inducing SHP-1 in hepatocellular carcinoma cells,[12] inhibit the activation of PI3K/Akt pathway, phosphorylation of PTEN and mammalian target of rapamycin (mTOR), and the activity of cAMP-dependent protein kinase A (PKA) in pancreatic cancer,[13] inhibit JNK- and PERK-mediated phosphorylation of the Bcl-2 protein leading to the disruption of mitochondrial membrane potential in human renal carcinoma cells[5] and human ovarian cancer cells,[14] suppress ABCG2 mediated drug resistance by inhibiting p50/p65 NF-κB pathway in colorectal cancer[15] and so on. Besides, it could also sensitize chemoresistant breast cancer cells to adriamycin without obvious cytotoxicity against normal human peripheral blood cells.[16] Although evodiamine exhibited safe and known broad antitumor activity, the development of evodiamine for cancer therapy was stunted by its relatively moderate potency. Therefore, it is very necessary to develop novel evodiamine derivatives with improved antiproliferative activity by structural modification. To date, some evodiamine derivatives **A**–**F**[17–22] (Fig. 1) have been designed and synthesized, which also rouse our interest for further investigation.

Nitrogen mustards are DNA alkylating agents which have been widely used in cancer chemotherapy with the advantages of broad spectrum of antitumor activity and strong antiproliferative potency.[23] However, the lack of selectivity causes serious side effects, and acquired drug resistance limits the use of nitrogen mustards in clinic.[24] With this information in hand, it is necessary to carry out chemical modifications on the nitrogen mustards to enhance selectivity between normal and tumorous cells for the chemotherapy of cancer. Recent studies have underpinned that conjugating alkylating agents with an ideal natural compound of

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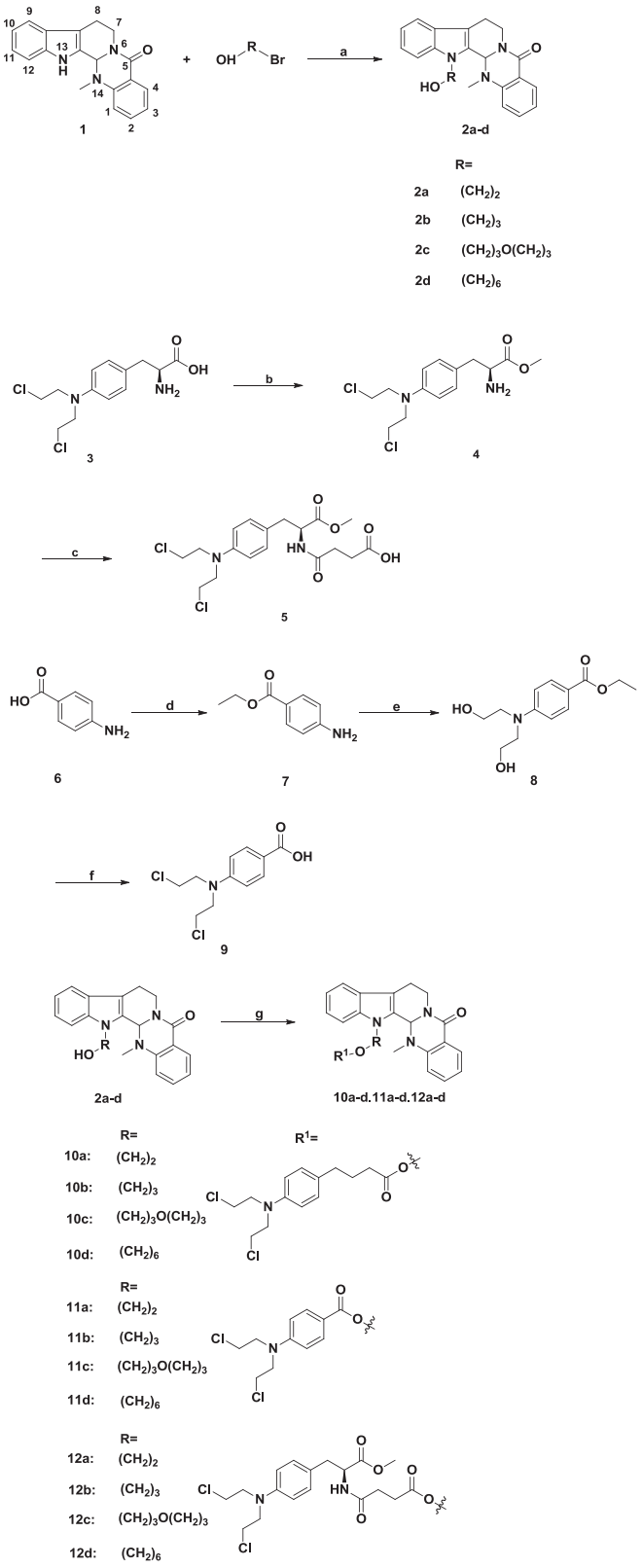

Scheme 1. Synthetic routine of target nitrogen mustard-evodiamine hybrids **10**–**12**. Reagents and conditions: (a) NaH, DMF, rt, 3 h; (b) $SOCl_2$, MeOH, reflux, 12 h; (c) succinic anhydride, DMAP, DCM, rt, 18 h; (d) H_2SO_4, ethanol, reflux, 6 h; (e) ethylene oxide, 25% aqueous acetic acid, rt, 24 h; (f) $POCl_3$, 50 °C, 0.5 h; 10% HCl, 12 h; (g) chlorambucil, 5 or 9, EDCI, DMAP, DCM, rt, 12–24 h.

special chemical scaffold is an attractive approach to enhance activity and reduce adverse side effects of nitrogen mustard drugs.[25,26]

Previously, we synthesized a series of nitric oxide (NO) donating derivatives of evodiamine[22] and verified that the introduction of the NO donating group to the *N*-13 position of evodiamine scaffold significantly enhanced antitumor activity. So, in this letter, we also designed and synthesized a series of evodiamine derivatives (bearing nitrogen mustards) at *N*-13 according to combinational principle. Then, the antiproliferative activities of target compounds were tested and preliminary mechanisms concerning the influence of cell cycle progression, effects on cellular apoptosis and mitochondrial membrane potentials by representative compound **12c** in HL-60 cells were also disclosed.

The synthesis routine of nitrogen mustard-evodiamine hybrids was illustrated in Scheme 1. First, **1** was treated with corresponding bromohydrin (2-bromine ethanol, 3-bromine-1-propanol or 6-bromine-1-hexanol) in the presence of NaH and anhydrous DMF as reported[19] to offer **2a**–**d** in high yields. Esterification of **3** in dry methanol in the presence of $SOCl_2$ led to ester **4**, and succedent reaction of **4** with succinic anhydride produced melphalan derivative **5** in quantitative yield**.** The benzoic acid mustard **9** was prepared according to the literature procedures.[27] At last, the target nitrogen mustard-evodiamine conjugates (**10a**–**d**, **11a**–**d** and **12a**–**d**) were synthesized from **2a**–**d** accordingly with nitrogen mustards (chlorambucil, **5** or **9**) in the presence of DMAP/EDCI in DCM at room temperature for 8–24 h. Each target compound[28] was purified by column chromatography (PE/EA 7:1–2:1 v/v) and confirmed by ^{1}H NMR, ^{13}C NMR and HR-MS (data in Supplementary Materials).

The antiproliferative activities of target hybrids **10**–**12** against four different kinds of human cancer cell lines (PC-3 human prostatic cancer cell line, HepG2 hepatocellular carcinoma cell line, THP-1 human acute monocytic leukemia cell line, and HL-60 human leukemic cell line) and human normal peripheral blood mononuclear cells (PBMC) were determined and compared with lead compound evodiamine (**1**), evodiamine intermediates (**2a**–**d**), nitrogen mustard compounds (chlorambucil, **5** and **9**) and positive control 5-fluorouracil (5-FU) in each panel (Table 1). Compounds **10c**, **12b** and **12c** were very sensitive to HL-60 cell lines with IC_{50} values ranging from 0.50 μM to 1.29 μM and even superior to 5-FU, and the other compounds were weaker than parent compound **1**. In THP-1 cell line, most hybrids showed the IC_{50} values at micromolar level, except for **11a**. While, in PC-3 and HepG2 cell lines, target compounds showed almost no cytotoxicity and only **12c** exhibited moderate activity against HepG2 cells with IC_{50} value of 17.04 μM. In PBMC cells, all the target hybrid compounds exhibited no antiproliferative activity (>200 μM). The antiproliferative selectivity between human tumorous and normal blood cells was a very important property for the further exploitation of antitumor drug. As shown in Table 1, generally, compounds **12a**–**d** incorporated with melphalan showed stronger activity than compounds **10a**–**d** and **11a**–**d** those with chlorambucil or benzoic acid mustard against THP-1 and HL-60 cells. When R were $(CH_2)_3$ and $(CH_2)_3$-O-$(CH_2)_3$ groups (**10b**–**c**, **11b**–**c** and **12b**–**c**), the antiproliferative activities were stronger than those of $(CH_2)_2$ and $(CH_2)_6$ groups (**10a**, **10d**, **11a**, **11d**, **12a** and **12d**). The derivative **12c** showed the most promising antiproliferative activity against HL-60 cells with IC_{50} value of 0.5 μM and selected for further investigation.

Cell cycle can be divided into four functional phases: S phase, when DNA replication occurred; M phase (mitosis), when DNA and cellular components are divided to form two daughter cells; G_2 phase, between S and M phases, when cells prepare for mitosis; G_1 phase, after mitosis and before S phase, when cells prepare for another round of DNA and cellular replication.[29] Many anticancer