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Original article

Design, synthesis and biological evaluation of novel sesquiterpene mustards as potential anticancer agents

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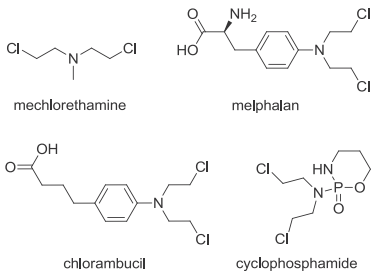
ABSTRACT

Several novel series of sesquiterpene mustards (**SM**s) bearing nitrogen mustard and glutathione (GSH)-reactive α-methylene-γ-butyrolactone groups were successfully prepared for the first time and showed excellent antiproliferative activities *in vitro*. Among them, compounds **2e** and **2g** displayed the highest antiproliferative properties with IC_{50} values ranging from 2.5 to 8.7 μM. The selectivity of these two compounds was evaluated by SRB method against human cancer and normal hepatic cells (HepG2 and L02). The induction of apoptosis and effects on the cell cycle distribution with compounds **2e** and **2g** were investigated by Hoechst 33,258 staining and flow cytometry, which exhibited that they could induce selective cell apoptosis and cell cycle arrest in HepG2 and L02 cells. In addition, further investigation showed that compounds **2e** and **2g** could obviously inhibit the proliferation of HepG2 cells by inducing significant DNA cross-linking and depleting GSH in cell media. The good cytotoxicity and selectivity of compounds **2e** and **2g** pointed them as promising leads for anticancer drug design.

1. Introduction

Nitrogen mustards are the most powerful alkylating agents widely used in the chemotherapy. Their anticancer ability has been ascribed to the formation of interstrand and intrastrand cross-links by coordinating to N (7) of purine bases through aziridine cation *in vivo*. Mechlorethamine is one of the oldest synthetic anticancer drugs and used in a combination chemotherapy with oncovin (vincristine), procarbazine and prednisone (MOPP) for the treatment of Hodgkin's disease [1]. The alkylating agent melphalan (phenylalanine mustard) has been a key component in the treatment of multiple myeloma for several decades [2,3]. Also, other derivatives, such as chlorambucil (Leukeran) [4–6] and cyclophosphamide [7–9], are well known to be used to treat cancer in clinic.



However, these drugs are not selective toward neoplastic cells and produce undesirable effects, such as bone marrow depression and

Abbreviations: **SM**s, sesquiterpene mustards; HepG2, human hepatocarcinoma cells; L02, human normal hepatic cells; GSH, glutathione; TBHP, *t*-butylhydroperoxide; DEAD, diethylazodicarboxylate; NEM, *N*-ethylmaleimide; DCM, dichloromethane.

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typical clinical signs including erythema, blister formation and inflammation occurring after a characteristic latency period (up to 24 h), depending on the concentration and amount of the alkylating agent [10].

To increase the efficacy of the therapy, many researchers have focused on changing the carrying parts for nitrogen mustards, such as 2-deoxy-2-fluoro-D-glucose (FDG) [11], steroids [12], distamycin [13,14], polyamine [15], polyazamacrocycles [16] and benzene derivatives [17–19]. In another strategy, the cytotoxic agent is given in its prodrug form, which is safe to normal cells but selectively activated by certain biochemical mechanisms unique to tumor cells, such as active oxygen species (ROS) [20] and hypoxia [17], resulting in localized cytotoxicity in tumor tissues. However, most efforts have been so far not so significant and there is considerable scope for developing nitrogen mustard drugs with reduced side effects and improved selectivity.

GSH is the most abundant nonprotein thiol in the cells [21] and involved in physiologically relevant metabolic functions as well as cell protection [22]. Depletion of GSH was known to induce growth inhibition and apoptosis through various pathways [23–26]. Moreover, a number of *in vitro* studies have shown that agents which deplete GSH can restore sensitivity to alkylating agents and then increase the cytotoxicity of alkylating agents toward cancer cells [27–31]. In another way, the level of GSH in cancer tissues is apparently higher than in normal tissues [32,33] and only can satisfy the need of tumor cell metabolism, which makes the selective growth inhibition on cancer cells possible. In addition, natural products **a** and **b** (Fig. 1) with α-methylene-β-lactone group have been proved to efficiently deplete GSH [34,35] and show a diverse range of biological activities, including anticancer [36,37], anti-inflammatory [38], antibacterial [39] and fungicidal properties [40]. Also, they can easily penetrate the cell membrane into the cell. Therefore, we introduced them to nitrogen mustards by esterification in order to obtain bifunctional antitumor agents with enhanced antiproliferative activities and selectivity. In this paper, several novel series of **SM**s were synthesized and evaluated for antiproliferative activities by SRB assay. The results indicated that most of them showed diverse but strong antiproliferative effects. The results of our series of biological experiments demonstrate that both **2e** and **2g** have a high degree of selectivity for cancer and normal cells and are promising leads for the development of new anticancer agents.

2. Results and discussion

2.1. Chemistry

Dehydrocostuslactone (**a**) and costunolide (**b**) were obtained from crude costus resin oil (*Saussurea lappa*) by column chromatography (CC) separation. The sesquiterpenes **c** and **d** were prepared according to the reported approach [41,42]. When the reaction time was raised to 12 h, the sesquiterpene **e** was obtained with 48.5% yield (Scheme 1). Slight modifications to the procedure gave the sesquiterpene derivatives **f**(30%) and **g**(10%) (by replacing $CHCl_3$ with CH_2Cl_2 and the starting material **a** with **b**, Scheme 1)

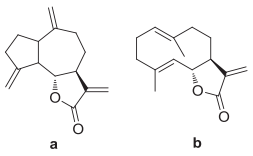
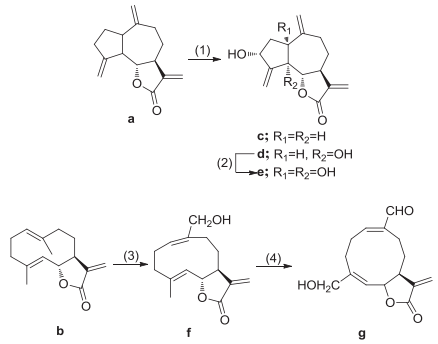


Fig. 1. Structures of dehydrocostuslactone (**a**) and costunolide (**b**).



Scheme 1. Synthesis of sesquiterpenes. Reagents and conditions: (1) 0.1 M in $CHCl_3$, **a**/SeO_2/TBHP (1:1:4), 2 h, rt; (2) 0.1 M in $CHCl_3$, **d**/SeO_2/TBHP (1:1:4), 12 h, rt; (3) CH_2Cl_2, **b**/SeO_2/TBHP (2:1:4), 24 h, rt; (4) CH_2Cl_2, **f**/SeO_2/TBHP (2:1:4), 8 h, reflux.

[43,44].

The route for the synthesis of nitrogen mustards **x**(**x** = **1** − **5**) was outlined in Scheme 2. The synthesis of these compounds required the starting materials **x**(**a**) (**x** = 1, 2, 3, 4, 5). The subsequent esterification by ethanol afforded the esters **x**(**b**), which were converted in acceptable yield to the corresponding *N*, *N*-bis(2-hydroxyethyl) derivatives **x**(**c**) by reaction with excess of ethylene oxide in acetic acid. Subsequent treatment with phosphorus oxytrichloride ($POCl_3$) afforded the corresponding nitrogen mustards, which were transformed into the desired acids **1**–**5** by acid hydrolysis.

The desired **SM**s (**1c**–**g**, **2c**–**g**, **3c**–**g**, **4c**–**g**, and **5c**–**g**) were prepared by esterification of the corresponding sesquiterpenes (**c**–**g**) with mustards (**1**–**5**) in the presence of dry CH_2Cl_2 at 40 °C. Starting from sesquiterpenes **d** and **e**, compounds **2d′** and **2e′** with α-orientation of H-3 were performed by catalysis of diethylazodicarboxylate (DEAD) with 50.0% and 69.7% yield (Scheme 3). These **SM**s were confirmed by spectroscopic methods: ^{1}H NMR, ^{13}C NMR as well as ESI-MS. Finally, bis(2, 4-dinitrobenzenesulfonyl) fluorescein was prepared according to a reported procedure as fluorescent probe imaging free thiols (mainly GSH) [45].

2.2. Evaluation of biological activities

2.2.1. Antiproliferative activities

The antiproliferative effects of novel **SM**s were evaluated *in vitro* against four human cancer cell lines (SMMC-7721, HepG2, A549 and HeLa) using SRB method. Chlorambucil (Chlo.) was used as positive control. The concentrations that induce 50% inhibition of cell growth (IC_{50}) in μM are reported in Table 1. Results showed that the tested compounds showed diverse but mostly strong antiproliferative effects compared with the parent drug (chlo., **a** and **b**).

Initial structure-activity relationship studies for **SM**s focused on the carrying groups (structures and configuration of sesquiterpenes). Guaiane sesquiterpene mustards (**1**–**5**(**c**), **1**–**5**(**d**) and **1**–**5**(**e**)) were first explored. Results showed that they exhibited good cytotoxicity except compound **1e** (12–37 μM). However, there was some slight difference between them. Compounds **4d** and **4e** ($4.5 \leq IC_{50} \leq 10.2$, $7.2 \leq IC_{50} \leq 15.9$), for example, were more potent than **4c** ($6.9 \leq IC_{50} \leq 19.0$). A similar tendency was also observed by comparison of other **xd** and **xe** with the compounds **xc** (**x** = **1**, **2**, **3** and **5**), which suggested that the introduction of the hydroxyl group significantly improved the antiproliferative activities. Moreover, the effect of the configuration of H-3 was also evaluated. The results clearly showed that compounds **2d′** and **2e′** with H-3 in α-orientation exhibited poor antiproliferative abilities (at least 2-