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Original article

Design, synthesis and *in vitro* evaluation of novel dehydroabietic acid derivatives containing a dipeptide moiety as potential anticancer agents

Xiao-Chao Huang [a, 1], Le Jin [a, 1], Meng Wang [a], Dong Liang [a], Zhen-Feng Chen [a], Ye Zhang [a, b], Ying-Ming Pan [a, *], Heng-Shan Wang [a, *]

[a] State Key Laboratory Cultivation Base for the Chemistry and Molecular Engineering of Medicinal Resources, School of Chemistry and Pharmaceutical Science of Guangxi Normal University, Yucai Road 15, Guilin 541004, Guangxi, PR China
[b] Department of Chemistry and Pharmaceutical Science, Guilin Normal College, Xinyi Road 21, Guangxi 541001, PR China

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ABSTRACT

A series of novel dehydroabietic acid (DHA) chiral dipeptide derivatives were designed and synthesized as potent antitumor agents. The inhibitory activities of these compounds against NCI–H460 (lung), HeLa (epithelial cervical) and MGC-803 (gastric) human cancer cell lines were estimated by MTT assay *in vitro*. The antitumor activities screening indicated that many compounds showed moderate to high levels of antitumor activities against these three cancer cell lines and most of these compounds displayed more potent inhibitory activities compared with commercial anticancer drug 5-fluorouracil (5-FU). The induction of apoptosis and affects on the cell cycle distribution with compound **8k** were investigated by acridine orange/ethidium bromide staining, Hoechst 33258 staining, JC-1 mitochondrial membrane potential staining, TUNEL assay, flow cytometry and the activities of caspase-3 and -9 assay in Hela cells, which exhibited that the compound could induce cell apoptosis in Hela cells. In addition, further investigation showed that apoptosis were associated with loss of mitochondrial membrane potential, enhancement of mitochondrial cytochrome *c* release and intracellular ROS production, elevation of Bax expression, down-regulation of Bcl-2, and the activation of caspase-9 and -3.

1. Introduction

Cancer, being one of the leading causes of death globally, is a disease of worldwide importance. As a result, there is a consist need of searching novel molecules with anticancer effective. Natural products played an important role in drugs discovery, especially in the area of cancer pharmacology. They are still major source of new antitumor drugs development, and many natural or natural based antitumor drugs such as taxol, vinblastine, bleomycin and doxorubicin derivatives were clinically used in recent year [1–3]. Encouraged by these research results, our interest in investigating natural products for their potential therapeutic effects has recently spurred us to examine the influences of dehydroabietic acid derivatives on antitumor properties.

DHA is a natural occurring diterpenic resin acid, which can be easily isolated from commercial disproportionated rosin [4]. Recent reports indicate that DHA and its derivatives exhibited wide range of biological activities, such as antiulcer, antimicrobial, antifungal, anti-inflammatorily, anti-pepsin, anxiolytic, antiviral, antitumor, and cytotoxic activities [5–11]. Modern studies have indicated that DHA and some derivatives have been reported to have anticancer activity in many human cancer cells such as cervical carcinoma cells, hepatocellular carcinoma cells and breast cancer cells, as well as its analogs [12,13]. Previous work has also found that the introduction of functional groups ureas in carboxylic acid group of DHA would improve antitumor activity [14]. Furthermore, in our previous work, it has found that the introduction of functional groups thiourea or/and α-aminophosphonate groups in carboxylic acid group of DHA showed improved antitumor activity [15]. As a result, our present work in this paper is to design and synthesize a new class of DHA derivatives, and to evaluate their *in vitro* anticancer activities.

Peptides play crucial roles in the human body and other organisms [16]. Previous work indicated that dipeptides and their

* Corresponding authors.
E-mail addresses: panym2013@hotmail.com (Y.-M. Pan), whengshan@163.com (H.-S. Wang).
[1] Co-first author: These authors contributed equally to this work.

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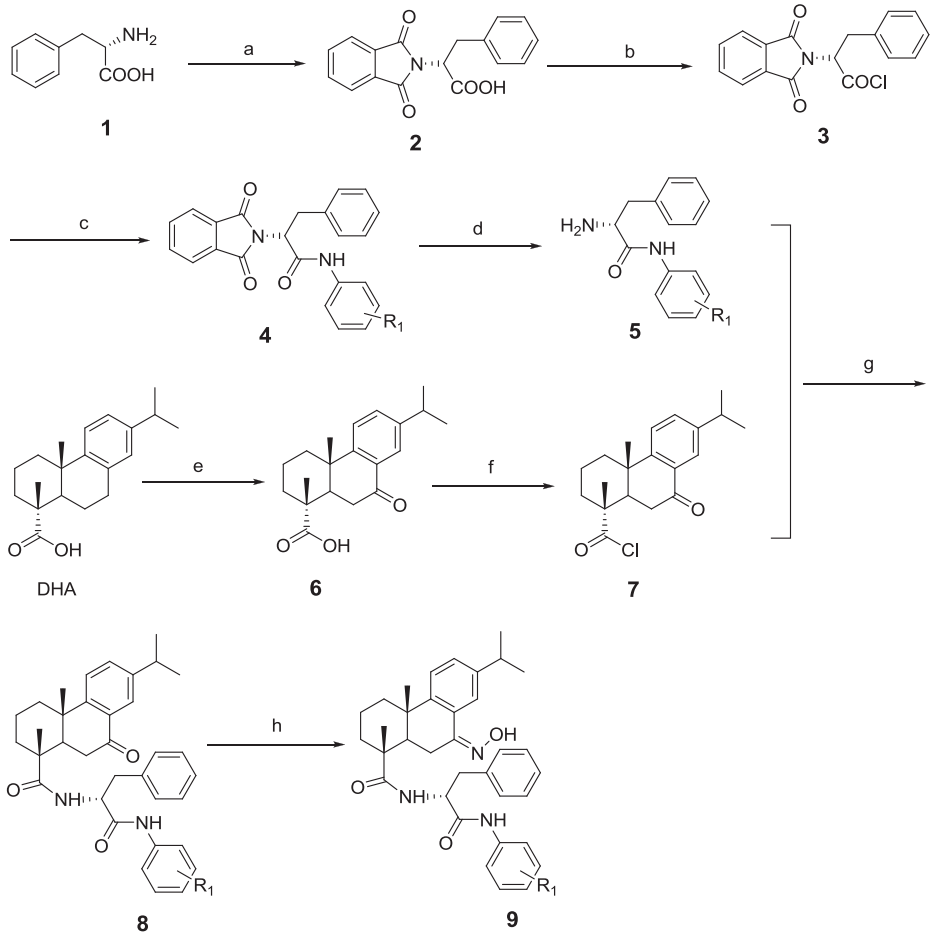
derivatives have exhibited a wide spectrum of important bioactivities such as antimicrobial, neuroprotective, antiviral and anticancer activities [17–19]. Peptides are among the most versatile bioactive molecules e.g. many peptide hormones and analogous short peptides exert their action by binding to membrane receptors [20,21]. However, most of natural peptides consists of L-form α-amino acids and due to the ubiquitous prevalence of peptidases, they exhibit limited biostability, and consequently low bioavailability [21]. To solve this problem, other stable and biologically active peptides have been thus developed. Several L- and D-amino acids have been thus introduced into the natural products skeleton, and such analogs as conjugates of paclitaxel, doxorubicin and daunorubicin with an amino acid or peptides have been demostrated increased and more selective anticancer activity than the drugs themselves [22–26]. In addition, previous work demonstrated that many oxime derivatives exhibited good antitumor activity and the introduction of oxime group to some active core skeleton may lead to better antitumor activity [27,28]. So in this paper, as a development of the previous research work [29], we have designed and synthesized a series of new DHA dipeptide derivatives containing oxime group. Their cytotoxicities *in vitro* against three selected tumor cell lines were evaluated. Results showed that the target compounds can inhibit proliferation on these three tumor cell lines at moderate to high rates. Moreover, our results clearly demonstrated that compound **8k** can induce apoptosis in HeLa cells. Furthermore, the related molecular mechanism involving the apoptosis effects induced by **8k** was also investigated.

2. Results and discussion

2.1. Chemistry

A new class of novel DHA chiral dipeptide derivatives were synthesized as outlined in Scheme 1. The synthetic route to the targeted molecule is simple, concise, and high yielding. Compound **2** was synthesized by the treatment of phenylalanine **1** with phthalic anhydride in the presence of acetic acid according to the literature [30]. Compound **3** was then obtained by the condensation of compound **2** and oxalyl chloride, and it was then treated with series of aromatic primary amines to offer compounds **4**. Compounds **5** were synthesized by the treatment of compounds **4** with hydrazine hydrate in the presence of ethanol at room temperature. 7-Oxo-dehydroabietic acid **6** was synthesized by the treatment of dehydroabietic acid with chromic anhydride in the presence of glacial acetic acid [31]. 7-Oxo-dehydroabietic acid was treated with oxalyl chloride to offer compound **7**. Compounds **8** were finally acquired by the condensation of compound **7** and compounds **5** in the presence of triethylamine at room temperature. Compounds **9** were finally acquired by the condensation of compounds **8** with hydroxylamine hydrochloride in the presence of ethanol at 80 °C. The structures of DHA dipeptide derivatives **8**–**9**



Scheme 1. Synthetic pathway to target compounds **8a**–**8p** and **9a**–**9p**. Reagents and conditions: (a) phthalic anhydride, CH_3COOH, 50 °C; (b) oxalyl chloride, CH_2Cl_2, r.t.; (c) aromatic primary amines, Et_3N, CH_2Cl_2, r.t.; (d) hydrazine hydrate, CH_3OH; (e) CrO_3, CH_3COOH, r.t.; (f) oxalyl chloride, CH_2Cl_2, r.t.; (g) Et_3N, CH_2Cl_2, r.t.; (h) hydroxylamine hydrochloride, CH_3CH_2OH, 80 °C.