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Research paper

Synthesis and pharmacological evaluation of dehydroabietic acid thiourea derivatives containing bisphosphonate moiety as an inducer of apoptosis

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ABSTRACT

A series of DHAA thiourea derivatives containing bisphosphonate moiety were designed and synthesized as potent antitumor agents. Structures of target molecules were confirmed using HR-MS, ^{1}H NMR and ^{13}C NMR and they exhibited potent anti-tumor activities against the SK-OV-3, BEL-7404, A549, HCT-116 and NCI-H460 tumor cell lines *in vitro*. Especially, compound **6e** (IC_{50} = 1.79 ± 0.43 μM) exhibited the best anticancer activity against SK-OV-3 cell line. Its role as an inducer of apoptosis was investigated in this cell line by Annexin-V/PI binding assay and by following its capability for ROS generation, depolarization of mitochondrial transmembrane potential, activation of caspases and expression of pro- and anti-apoptotic proteins. Elevated level of ROS generation, activation of caspase-3, caspase-8, caspase-9, and Fas, higher expression of Bax, lower expression of Bcl-2, and increased level of Bax/Bcl-2 ratio identified **6e** as a promising inducer of apoptosis that follows both of the mitochondria dependent pathway and the death receptor-mediated pathway. In addition, the cell cycle analysis indicated that compound **6e** caused cell cycle arrest at G1 phase, induced apoptosis and led to cell death by increasing the proportion of sub-G1 cells. Furthermore, molecular docking studies showed that **6e** could bind to the ATP pocket sites.

1. Introduction

As one of the leading causes of death globally, cancer is characterized by uncontrolled cell proliferation and will become a global health problem. Natural products are a rich source of treatments for cancers, such as doxorubicin, paclitaxel, vinblastine and their derivatives were used clinically in recent years [1,2]. In fact, at least 60% of anticancer agents originate from natural compounds [3]. Dehydroabietic acid (DHAA), a natural occurring diterpene rosin acid, which can be easily isolated from commercial disproportionated rosin. DHAA and its derivatives exhibit a broad spectrum of biological action, such as antiulcer, antimicrobial, anxiolytic, antiviral, antitumor, and cytotoxic activities [4–8]. Recent reports indicated that DHAA and their derivatives have anticancer activity in many human cancer cells such as cervical cancer cells, hepatocellular cancer cells and breast cancer cells [9]. Our previous study has also demonstrated that the introduction of α-aminophosphonate and dipeptide moiety in carboxylic acid group of DHAA showed increased anticancer activity [10].

Recently, bisphosphonates (BPs) have been proven to be an important asset in the treatment and prevention of bone metastatic breast cancer [11–16]. A number of *in vitro* studies indicated that BPs has remarkable anti-tumor effects through the induction of tumor cell apoptosis and the inhibition of tumor cell proliferation, invasion and adhesion to bone [17,18]. Moreover, preclinical studies have demonstrated the anticancer activity of bisphosphonates *in vitro* and *in vivo*. The urea and thiourea derivatives play important role in anticancer agents because of their good inhibitory activity against malignant tumor [19–21]. Previously, we have synthesized a novel class of DHAA thiourea α-aminophosphonates

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derivatives which exhibited potent anticancer activities against the tested cancer cell lines *in vitro* [22]. In light of these research results, our present work in this paper is to design and synthesis a series of new DHAA thiourea derivatives containing bisphosphonate moiety. Their cytotoxicities *in vitro* against the SK-OV-3, BEL-7404, A549, HCT-116 and NCI-H460 tumor cell lines were evaluated. Furthermore, the molecules mechanism of apoptotic pathway induced apoptosis in SK-OV-3 cells by the representative of the target compound **6e** is also investigated.

2. Results and discussions

2.1. Chemistry

DHAA thiourea derivatives containing bisphosphonate moiety were synthesized as outlined in Scheme 1. DHAA was treated with oxalyl chloride to offer compound **1**. Compound **1** was then treated with KSCN to offer compound **2**. Compounds **4** were finally acquired by the condensation of compound **2** and compounds **3** in CH_2Cl_2 at room temperature. Compound **5** was prepared according to the reported procedure [23]. The compound **5** on addition reaction conditions at room temperature with compounds **4** gives target compounds **6**. The structures of DHAA thiourea derivatives containing bisphosphonate moiety derivatives **6** were confirmed by ^1H NMR, ^{13}C NMR and high resolution mass spectrum (HR-MS).

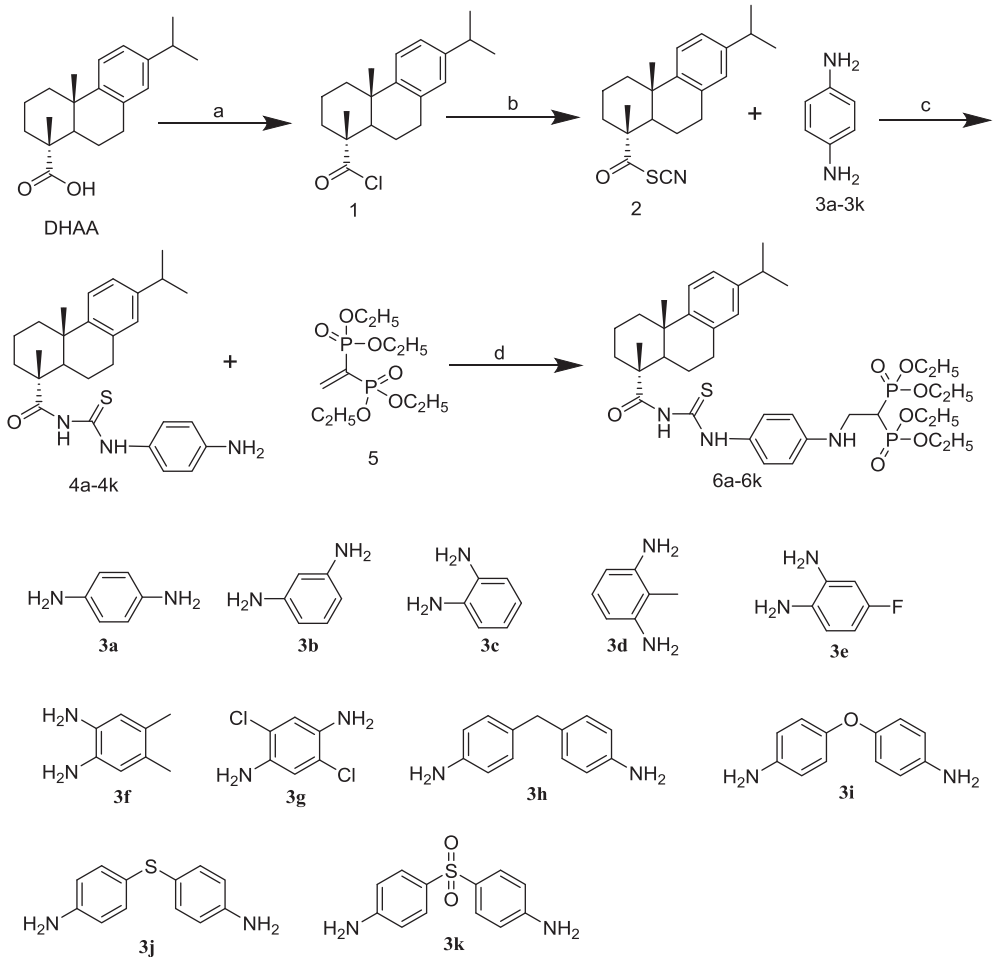
2.2. Biological evaluation

2.2.1. Cytotoxicity test

The *in vitro* cytotoxic potency of test compounds **6a**–**6k** were evaluated by MTT assay against SK-OV-3, BEL-7404, A549, HCT-116 and NCI-H460 tumor cell lines, with 5-FU as the positive control. The tested results were shown in Table 1.

As shown in Table 1, most of test compounds exhibited much higher inhibitory activity than DHAA against the SK-OV-3, BEL-7404, A549, HCT-116 and NCI-H460 tumor cell lines, indicating the introduction of bisphosphonate moiety on DHAA could markedly increase the anti-tumor activity. Moreover, target compound **6a**–**6g** showed higher inhibitory activity than **6h**–**6k**, DHAA and 5-FU against the test human cancer cell lines.

Table 1 also revealed that, In SK-OV-3, BEL-7404, A549 HCT-116 and NCI-H460 cell lines assay, all compounds displayed better cytotoxicity than DHAA, and most of compounds displayed preferable cycotoxic activities than the commercial anticancer drug 5-FU. Compounds **6a**–**6c** showed similar inhibitor activities and exhibited better cytotoxic inhibition and substituent of group methyl at 2-position of phenyl ring of compound **6d**, with IC_{50} in the range of 11.94–17.28 μM showed decrease of cytotoxic inhibition than compound **6b** (IC_{50} = 11.32–16.49 μM). The substituent of group fluorine at 4-position of phenyl ring of compound **6e** exhibited the best cytotoxicity activities against the test human cancer cell lines, with IC_{50} in the range of 1.79–8.17 μM, even



Scheme 1. Synthetic pathway to target compounds **6a**–**6k**. Reagents and conditions: (a) $C_2O_2Cl_2$, CH_2Cl_2, r.t.; (b) $C_6H_5CH_3$, KSCN, 110 °C; (c) CH_2Cl_2, r.t.; (d) DMAP, CH_2Cl_2, r.t.