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Short communication

Click chemistry-based synthesis and anticancer activity evaluation of novel C-14 1,2,3-triazole dehydroabietic acid hybrids

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ABSTRACT

A concise and efficient synthetic approach has been established to readily access a series of novel C-14 1,2,3-triazole-tethered dehydroabietic acid derivatives in moderate to high yields. *In vitro* antiproliferative activity evaluation indicated that most of the hybrids exhibited potent inhibitory activities in a variety of cancer cell lines with low micromolar to submicromolar IC_{50} values. Further studies demonstrated that some of these analogues such as **20**, **21**, and **24** were also effective against adriamycin-resistant MCF-7 clone at low concentrations in a dose-dependent manner. Notably, the most potent compound **24**, which possesses a 3-(*tert*-butoxycarbonylamino)phenyl-substituted triazole moiety, not only exhibited obviously improved IC_{50} values ranging from 0.7 to 1.2 μM against a panel of tested cancer cells, but also showed very weak cytotoxicity on normal cells. Preliminary mechanism studies indicated that compound **24** could induce apoptosis in MDA-MB-231 cells and was worth developing into a novel natural product-like anticancer lead by proper structure modification.

1. Introduction

Cancer is characterized by its high incidence and mortality [1]. Despite the availability of various chemotherapeutic drugs, the rapid development of multidrug resistance (MDR) and associated acute side effects of clinical used anticancer drugs (including the recently introduced drugs) are still the major obstacles to effective cancer chemotherapy [2]. Consequently, the discovery and development of new effective anticancer agents and/or therapies to address the unmet demands is always the major goal of medicinal chemists. On the other hand, natural products (NPs) are structural-complex molecules that have a profound impact upon medicinal chemistry. Their diversity three-dimensional shape, functionality, stereochemistry, as well as various interesting biological activities have always provided medicinal chemists a reliable source of inspiration in their search for new drug-like molecules [3]. It is reported that approximately 60% of clinically used antitumor drugs originated from NPs. For example, vincristine, vinblastine, irinotecan, etoposide, doxorubicin and paclitaxel are well-known naturally derived chemotherapeutic agents in clinical use [4].

Terpenoids are the most abundant and widely distributed naturally occurring interesting metabolites. As the largest class of NPs, it contains approximately 25,000 chemical structures, thus exhibiting extensive pharmaceutical activities [5]. Among them, abietic acid (AA, **1**) and dehydroabietic acid (DHAA, **2**) are natural resin acids which can be easily obtained from pinus rosin or disproportionated rosin (Fig. 1). It is reported that AA, DHAA and their derivatives showed a broad spectrum of bioactivities such as anti-inflammatory, antiviral, antimicrobial, BK channel-opening and especially antitumor activities [6]. As a result, AA and DHAA have been widely used as the starting material for the construction of several NPs and structurally diverse natural product-like (NPL) molecules library [7].

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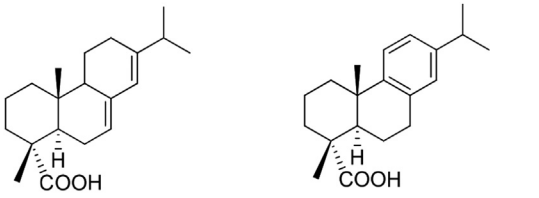

Abietic acid (AA) (1) Dehydroabietic acid (DHAA) (2)

Fig. 1. Structures of abietic acid (AA) (**1**) and dehydroabietic acid (DHAA) (**2**).

In general, the search for new drugs based on structural modification of NPs has often been hindered by the tedious and complicated synthetic pathways [8]. "Click chemistry", a concept initiated by Barry K. Sharpless [9], provided a nearly perfect strategy to perform selective modification on structural complex NPs under mild conditions, even in the presence of reactive functionalities. Of particular interest is the copper(I)-catalyzed alkyne-azide [3 + 2] cycloaddition (CuAAC) reaction, which provides 1,4-disubstituted 1,2,3-triazoles [10]. This well-known pharmacophore is quite resistant to metabolic degradation and capable of participating in dipole-dipole interactions as well as hydrogen bonding, thus providing additional advantages including cell permeability improvement and target binding [11].

Considering the above benefits and in continuation of our interest in searching for the anticancer pharmacological effects of abietane diterpenoids derivatives [12], we therefore conducted a modification via integrating dehydroabietic acid (DHAA) and triazole pharmacophore into one molecule to generate a novel C-14 1,2,3-triazole dehydroabietic acid hybrid scaffold through CuAAC for further biological evaluation [13]. Herein, we described the synthesis of these hybrids and the evaluation of their *in vitro* antiproliferative activities, selectivities over normal human cells, potential against drug-resistance tumor cells, *in vitro* metabolic stabilities and preliminary elucidation of their underlying mechanisms of cytotoxicity.

2. Results and discussion

2.1. Chemistry

The synthetic route towards the target DHAA-1,2,3-triazole hybrids was depicted in Scheme 1. Briefly, the key intermediate C-14 azide acetamino-DHAA **8** was synthesized from DHAA by a five-step streamlined manipulation. First, esterification of **2** provided the corresponding ester **3** in 98% yield. Subsequent nitration of ester **3** afforded a 3:2 mixture of **4** and **5**. Reduction of **5** followed by amidation of the resulting C14-amine with chloracetyl chloride delivered **7**, which was treated with sodium azide to give the key intermediated **8** in high yield. Finally, reaction of **8** with various selected aliphatic and aromatic alkynes in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate produced target derivatives **9**–**34** in moderate to high yields. Further, preliminary drug-likeness properties of the newly synthesized molecules were determined by in silico Molinspiration online property calculation software and the data obtained were presented in Table 1 [14]. For most compounds, the parameters like hydrogen bond acceptor (*n*-ON), hydrogen bond donor (*n*-OHNH), rotatable bonds (*n*-rotb) were in the range of Lipinski's rule of 5 [15]. As expected, compounds with trimethyl chlorosilane- (**13**), benzyl- (**14**) and substituted phenyl-on the C-4 position of the 1,2,3-triazole moiety showed higher lipophilicity (LogP 4.39–6.99), while compounds with saturated alkyl- (**10** and **11**), ester- (**12**), pyridyl- (**30**, **31** and **32**) and thienyl-possessed lower lipophilicity (LogP 3.67–5.12). Morever, the polar surface area (TPSA) of all the compounds (**9**–**34**) were less than 140 A^2 [16].

2.2. Biological assays

From the synthetic route mentioned above, we obtained a series of novel DHAA-1,2,3-triazole hybrids. To examine whether the substituents affected their cytotoxic activities, all the synthesized hybrids were evaluated for their potential against four different human tumor cell lines including ovary (SKOV-3), prostate (PC-3) and breast (MDA-MB-231 and MCF-7) by using MTT assay. 5-Fu and triptonide were used as positive controls for comparison [17].

As expected, most of the hybrids exhibited moderate to high cytotoxic activities against four tumor cell lines with IC_{50} values below 10 μM (Table 1). Among them, hybrids **20**, **21** and **24** displayed significant cytotoxic activities against all the tested cancer cells with IC_{50} values ranging from 0.7 ± 0.1 to 1.9 ± 0.2 μM. Notably, compound **24** showed the strongest activity against MDA-MB-231 cells with an IC_{50} of 0.7 ± 0.1 μM, indicating a higher potency compared with the commercial anticancer drug 5-Fu. Meanwhile, the cytotoxic activities of the intermediates **6**, **7** and **8** were also examined. The results revealed that **6** and **7** only displayed moderate cytotoxic activities, suggesting that the introduction of 1,2,3-triazole moiety was beneficial to cytotoxicity.

Further, the effect of various substituents on the C-4 position of the 1,2,3-triazole moiety was also examined and the structure-activity relationship (SAR) studies revealed that the introduction of aromatic substituents was crucial for the potent cytotoxicity. Generally, the introduction of electron-rich aromatic ring system could significantly increase the cytotoxicity. In particular, compounds **19**, **20**, **21** and **24** showed promising IC_{50} values ranging from 1.0 to 2.3, 0.8 to 1.4, 1.4 to 1.9 and 0.7–1.2 μM respectively. Whereas, the introduction of electron-poor aromatic ring system would hamper the cytotoxicity. For example, pyridyl- (**30**, **31** and **32**) and nitrobenzyl-substituted analogues (**25** and **26**) only showed weak or loss of cytotoxicities. In comparison, the saturated alkyl- (**10**, **11**, and **33**), ester- (**12**) and trimethyl chlorosilane- (**13**) on the C-4 position of the 1,2,3-triazole moiety were only associated with moderate increase in the growth inhibitory effect.

On the basis of the results above, we further evaluated the *in vitro* antiproliferative activity of the most potent compound **24** against another six tumor cell lines, which derived from different human tissues, including glioma (U251), breast (SKBR-3), lung (A549), colon (HCT-116), hepatoma (SMCC-7721) and prostate (LNCaP). The result showed that compound **24** strongly inhibited the growth of all the tested cancer cells, exhibiting IC_{50} values ranging from 0.6 to 1.8 μM (Table 2). Meanwhile, the IC_{50} values in normal human foreskin fibroblast (HFF) and human liver (HL-7702) cells were 5.4 μM and 7.8 μM respectively, implying there was a therapeutic window to use compound **24**.

In principle, drug resistance to chemotherapy remains to be a major impediment to the successful treatment of cancer. Therefore, the discovery of new chemical entities with the ability to overcome the drug resistance in tumor cells is likely to improve the therapeutic index. To investigate whether the thiazole-tethered DHAA derivatives synthesized above were still effective on drug resistant cancer cell lines, the most potent derivatives **20**, **21**, and **24** were selected for further evaluation of their potential on human breast adriamycin-resistant MCF-7 (MCF7/ADR) cells at 0.5, 2.5, and 5 μM concentration. The results showed that all of them exhibited significant inhibitory effect on MCF-7/ADR cells (Fig. 2) and the growth inhibition rates were even greater than 50% at 2.5 and 5 μM. Particularly, compound **24** with a 3-(*tert*-butoxycarbonylamino) phenyl-substituent on the C-4 position of the triazole ring showed the highest potency on MCF-7/ADR cells with a submircomolar IC_{50} value (0.9 μM).