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Research paper

High anticancer potency on tumor cells of dehydroabietylamine Schiff-base derivatives and a copper(II) complex

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ABSTRACT

Five bioactive dehydroabietylamine Schiff-base derivatives ($\mathbf{L^1}$-$\mathbf{L^5}$) had been synthesized from Dehydroabietylamine ($\mathbf{L^0}$), and the complex $\mathbf{Cu(L^1)_2}$ had been obtained from the compound $\mathbf{L^1}$ and copper(II) acetate. Their activities against Hela (cervix), MCF-7 (breast), A549 (lung), HepG2 (liver) and HUVEC (umbilical vein, normal cell) *in vitro* were investigated. The toxicity of $\mathbf{L^1}$-$\mathbf{L^5}$ and $\mathbf{Cu(L^1)_2}$ was all lower than $\mathbf{L^0}$. For MCF-7 cell, $\mathbf{L^1}$, $\mathbf{L^3}$, $\mathbf{L^4}$, $\mathbf{L^5}$ and $\mathbf{Cu(L^1)_2}$ had higher antitumor activity than $\mathbf{L^0}$. The smallest IC_{50} value was 2.58 μM of $\mathbf{L^5}$. For A549 cell, the IC_{50} value of the compound $\mathbf{L^4}$ was smaller than $\mathbf{L^0}$, which indicated that the compound $\mathbf{L^4}$ had higher anti-A549 activity than $\mathbf{L^0}$. For HepG2 cell, the IC_{50} value of $\mathbf{L^4}$ (0.24 μM) and $\mathbf{L^5}$ (0.14 μM) were much smaller than $\mathbf{L^0}$, which suggested $\mathbf{L^4}$ and $\mathbf{L^5}$ had higher anti-HepG2 activity. $\mathbf{L^5}$ was 180 times more effective at inhibiting cultured HepG2 cells survival than normal cells, with average IC_{50} values of 0.14 and 25.56 μM. Furthermore, $\mathbf{L^0}$, $\mathbf{L^4}$ and $\mathbf{L^5}$ contrasting with Doxorubicin had been measured with the ability to induce apoptosis. It turned out that $\mathbf{L^4}$ and $\mathbf{L^5}$ could induce more HepG2 cells apoptosis, which suggested they may be potential antitumor drugs.

1. Introduction

In recent years, natural products have been extensively studied because of their pronounced antitumor, antibacterial and antifungal activities [1–9]. Among them, rosin has attracted more attention, due to a variety of products from rosin modification with excellent bioactivities. Dehydroabietylamine ($\mathbf{L^0}$) is one of important modified products of rosin.

Dehydroabietylamine and its derivatives all have been widely used in the fields of pesticide and pharmaceutical products, *etc* [10,11]. Recently, antitumor, antibacterial, antifungal, cytotoxic and antiparasitic activities of dehydroabietylamine derivatives are a focus of research in the forest chemical field. For example, Robertson and coauthors [12] concluded that dehydroabietylamine was 4.5 times more effective at inhibiting cultured melanoma cells survival than normal cells, with average IC_{50} values of 2 and 9.3 μM, respectively. Rao's group screened a series of imines, amides and ureas with a dehydroabietyl skeleton for their antitumor activities against SMMC7721 (liver), A549, C6 (glioma) and MCF-7 cancer cell lines with smallest IC_{50} values of 6.65, 0.75, 0.81 and 10.65 μM [13]. Lin studied the antitumor activities on Hey-1B ovarian cancer line for a series of Schiff base compounds derived from 12-amino-N-acetyldehydroabietylamine, with the IC_{50} values of 15–20 μg/mL [14]. Chen found the derivative N-benzoyl-dehydroabietylamine-7-one could possess androgen receptor binding activity with IC_{50} value of 83.8 μM [15]. Minni Pirttimaa found an amide of dehydroabietylamine and acrylic acid, which was highly potent against some parasites, such as showing an IC_{50} value of 0.37 μM against L. donovani axenic amastigotes [16]. Encouraged by these research results, our interest in investigating dehydroabietylamine derivatives for their potential therapeutic effects had recently spurred

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us to examine the influences of them on antitumor properties. In our work, a series of dehydroabietylamine Schiff-base derivatives had been synthesized and screened for their antitumor activities against Hela, HepG2, MCF-7, A549 and the toxicity of the cell HUVEC cancer cell lines.

Due to Schiff-bases transition-metal complexes' diverse biological and pharmaceutical activities, they have attracted lots of attention [17]. In the past, considerable effort has been made to explore new drugs based on transition-metal complexes, particularly, biocompatible copper(II) complexes [18–20]. Some copper(II) complexes with biological activities, such as antibacterial, antitumor and tumor-inhibiting properties, have been reported previously in the literature [18,21]. Palaniandavar and co-workers stated that copper(II) complexes are the best alternatives to cisplatin, because copper(II) is a biocompatible metal ion, and plays many important roles itself in biological systems [22,23]. Fei had screened two copper(II) complexes and the pyridine-based ligand for antitumor activities. When three complexes at a concentration of 50 μM, the inhibition rates of the two copper(II) complexes towards MCF-7, NCI–H460 (lung) and HepG2 were all higher than 79.2%, and all much higher than the ligand [24]. Ng found that a ternary copper(II) complex with 1,10-phenanthroline as ligand was effective against Hela, SKOV3 (ovarian), A549, PC9 (lung), Hone1, HK1, C666-1 (NPC), MCF-7, T47D (breast), Nalmawa, HL60 (lymphoma and leukemia) and SW480, SW48, HCT118 (colorectal) cancer cell lines with IC_{50} values (24 h) in the 1.7–19.0 μM range [25]. In our work, a new copper(II) complex **Cu(L^1)$_2$** had been prepared and antitumor activities had been investigated. The IC_{50} value suggested it had higher anti-MCF-7 and anti-A549 activities.

Many natural products used in cancer chemotherapy, including taxol [26], doxorubicin [27], VP16 [28], and camptothecin [29], have apoptosis-inducing activity. For example, Doxorubicin (DOX, also termed Adriamycin) is one of conventional drugs. DOX is widely used as a first-choice anticancer drug for many tumours and is one of the most effective anticancer drugs developed [30,31]. Millions of cancer patients have been treated with DOX, or its variants daunorubicin (Daun) and idarubicin (Ida) [31]. Apoptosis, the physiological mode of cell death, is related to the regulation of development and homeostasis. Plasma membrane blebbing, cell shrinkage, nuclear condensation, chromosomal DNA fragmentation, and formation of apoptotic bodies are included in its morphological characteristics. Many recent reports have indicated that many anticancer drugs or cancer chemopreventive agents act through the induction of apoptosis to prevent tumor promotion and progression. The process of apoptosis is made of different phases, including initiation, execution and degradation, and is activated by two major pathways. One is receptor-induced apoptosis that contains of the TNFR1, Fas (CD95), and TRAIL-receptors. In the so-called 'extrinsic' cell death pathway, the receptor (TNF-R1, Fas, and TRAIL) trimerizes, a cytoplasmic death domain forms and recruits TRADD and FADD. The other major route leading to apoptosis is the 'intrinsic' cell death pathway, activated by proapoptotic factors from the mitochondria, including cytochrome *c* and Apaf-1 [32,33]. We recently identified **L^4** and **L^5** also had apoptosis-inducing activity.

Our present study was to synthesize and screen high antitumor activity and low toxicity of dehydroabietylamine derivatives including organic compounds and metal coordination complexes. Among these derivatives, several were designed and synthesized as potent antitumor agents. The inhibitory activities of these compounds against Hela (cervix), MCF-7 (breast), A549 (lung), HepG2 (liver) 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 four cancer cell lines and most of these compounds displayed more potent inhibitory activities compared with dehydroabietylamine. The induction of apoptosis and affects on the cell cycle distribution with **L^4** and **L^5** were investigated by Annexin V-FITC/PI assay and flow cytometry in HepG2 cells, which exhibited that the compounds could induce cell apoptosis. The potent activities of these compounds and their relatively low toxicity make them attractive for the further development of new drugs.

2. Results and discussion

2.1. Chemistry

Commercially available (+)-dehydroabietylamine was used as a starting material to synthesize Schiff-base **L^1**–**L^5** by a facile method (Scheme 1). In order to study the effect of compound structures on biological activities, three salicylaldehydes with different functional groups and two imidazole aldehydes with different functional groups were designed to react with dehydroabietylamine to prepare five Schiff-base derivatives **L^1**–**L^5**. These reactions were in the similar condition with ethanol (AR) as solvent and acetic acid as catalyst. Usually many Schiff bases are not stable and in equilibrium with the corresponding aldehyde and amine. But, **L^1**–**L^5** are stable. One reason may be large space steric hindrance from aromatic diterpene structure with three rings. Another may be that **L^1**–**L^5** was in neutral to slightly alkaline conditions (pH 7.0–7.4.) during the experimental process. The main absorption peaks of the UV spectra did not change obviously within 6 days, which suggested the stability of **L^1**–**L^5**.

The yellow block-shaped crystal of **L^2** with monoclinic crystal system was obtained. **L^2** had two aromatic rings and two aliphatic rings. An aromatic ring with C(7), N(1), O(1) and H(1A) was coplanar, and the molecule displayed "L" type (Fig. 1). 10 carbon atoms formed the six-member aliphatic ring and the two rings appeared distorted chair conformation. The length of the new imine double bond C(7)–N(1) (1.2690(4) Å) is in agreement with the result of Lu (C(10)–N(2) 1.2913(3) Å) [34]. The CH=N resonance in compound **L^2** appeared at 8.29 ppm in the ^{1}H NMR spectrum and at 165.8 ppm in the $^{13}C\{^1H\}$ NMR spectrum respectively. The hydrogen bonds are shown in Table 1. Selected bond lengths and angles are presented in Table 2. The crystallographic data is shown in Table S1.

It's reported that copper(II) is a biocompatible ion and some copper(II) complexes had higher antitumor activities [18–20], so a copper(II) complex **Cu(L^1)$_2$** had been synthesized by the ligand compound **L^1** and copper(II) acetate in a mild condition with ethanol/H_2O as solvent. The structure of brown block-shaped single crystal **Cu(L^1)$_2$** contains a four-coordinate copper center in a distorted tetrahedral geometry which is coordinated by two nitrogen and two oxygen atoms of the Schiff-base ligands. The distorted tetrahedral geometry is formed by copper as the center, N(2) as the vertex and a bottom plane consisted of O(1), O(2) and N(1). The bond length between the copper and the salicylaldiminato oxygen atom Cu(1)–O(1) is 1.9212(18) Å, which is slightly longer than that of Cu(1)–O(2) (1.9027(15) Å). The bond angle of O(2)–Cu(1)–O(1) is 156.19(10)°, which is smaller than that of N(2)–Cu(1)–N(1) (164.17(10) °). The crystal structure of **Cu(L^1)$_2$** is shown in Fig. 2. Selected bond lengths and angles are presented in Table 2. The crystallographic data is shown in Table S1.

To verify the oxidation state of copper, XPS of **Cu(L^1)$_2$** was measured. The Fig. S1 presents the XPS Cu(2p) spectrum of the complex **Cu(L^1)$_2$**. The Cu(2p3/2) and Cu(2p1/2) peaks centered at 934.0 and 951.3 eV, respectively, were attributed to the presence of the Cu^{2+} chemical state as an indication for formation. Moreover, the shake-up satellite peaks of the Cu(2p3/2) and Cu(2p1/2) at 941.9 and 960.1 eV, respectively, confirmed formation of Cu^{2+} on