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Copper(II/I) complexes of 5-pyridin-2-yl-[1,3]dioxolo[4,5-*g*] isoquinoline: Synthesis, crystal structure, antitumor activity and DNA interaction

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ABSTRACT

Three new copper(II) complexes of 5-pyridin-2-yl-[1,3]dioxolo[4,5-*g*]isoquinoline (PYP), i.e. $[Cu_2(PYP)_2Cl_4]$ (**1**), $[Cu_4(PYP)_4(ClO_4)_2(H_2O)_2](ClO_4)_2\cdot 2H_2O$ (**2**), and $[Cu_2(PYP)_2Cl_4]_n$ (**3**), were synthesized and fully characterized. In comparison to free PYP, complexes **1**–**3** exhibited enhanced cytotoxicity against tested human tumor cell lines BEL-7404, SK-OV-3, A549, A375, MGC-803 and NCI-H460, with IC_{50} values ranging from 0.31 to 30.76 μM. Complexes **1**–**3** exhibited lower cytotoxicity to HL-7702 than them to cancer cells. Complex **1** induced apoptotic death of BEL-7404, which involved mitochondria in the process. Caspase-3 activation assay indicated that **1** could be an efficient activator of caspase-3. DNA binding studies by UV–vis, DNA-melting, competitive binding, CD, viscosity measurement and agarose gel electrophoresis, revealed that intercalation might be the most likely binding mode of **1** with DNA.

1. Introduction

Transition metal complexes with anticancer activity have been extensively studied following the discovery of cisplatin and its analogs as transcription inhibitors [1–3]. However, treatment failure is often caused by the development of side effects, drug resistance, etc [4]. As a result, it is necessary to design new metallodrugs that are less toxic and highly active in small dosages, especially against cell lines that have acquired high resistance to cisplatin. In this context, much attention has been drawn toward antitumor complexes based on non-platinum metals [5–9]. Ru(II) and Cu(II) complexes are regarded as the best alternatives to platinum-based complexes as anticancer agents [10,11]. Copper(II), which is known to play a significant role in biological systems, has also been used as a pharmacological agent [12,13]. A number of synthetic copper(II) complexes have been reported as potential anticancer agents both active in vitro and in vivo [14–18].

Isoquinolines, a type of alkaloids widely existing in traditional Chinese medicine, exhibit a variety of biological activities especially inhibition of cellular proliferation and cancer development [19–21]. For example, berberrubine, a protoberberine alkaloid, shows antitumor activity in animal models [22]. Most of aporphine and oxoaporphine alkaloids which contain isoquinoline skeleton exhibit antitumor activity [23]. Various bioactivities have been found in [1,3]dioxolo[4,5-*g*]isoquinoline (papraline), a typical isoquinoline alkaloid isolated from the aerial parts of *Fumaria indica* which is regarded as a laxative, diuretic, alterative and mild analgesic, beneficial for dyspepsia and scrofulous skin infections [24,25]. Moreover, 2-substituted isoquinolines with aryl have shown enhanced anticancer activity [21]. Because of the extensive biological effects of isoquinolines, a series of platinum(II) complexes of isoquinoline and derivatives with potent anticancer activity have been reported by Osella and Farrell et al. in the past two decades [26–28]. However, very few examples of isoquinoline as chelating, nonleaving ligands in cis-platinum(II) antitumor complexes have been documented [29]. Previously, we reported promising anticancer activity in transition metal complexes of

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isoquinoline alkaloids having the active ingredients liriodenine and oxoglaucine found in traditional Chinese medicine [30–32]. To further our search for new metal-based anticancer agents, we designed and synthesized a new isoquinoline derivative, 5-pyridin-2-yl-[1,3]dioxolo[4,5-*g*]isoquinoline (PYP), with papraline as the leading compound. PYP contains a DNA intercalation moiety and a pyridine ring. It can act as a chelating, nonleaving ligand (Scheme 1). Herein, we report the synthesis, crystal structure and in vitro antitumor activity of three copper(II/I) complexes with the chelating ligand PYP. The mechanism of their cytotoxicity and their interactions with DNA were also investigated.

2. Results and discussion

2.1. Synthesis

Dihydroisoquinoline (MPDQ) was synthesized by Bischler–Napieralski cyclization of the acylation product (ALP) of phomopiperonylamine and picolinic acid. Then 5-pyridin-2-yl-[1,3]dioxolo[4,5-*g*]isoquinoline (PYP) was obtained by treating MPDQ with activated MnO_2 in toluene. Both were fully characterized by ESI-MS, ^1H NMR and ^{13}C NMR spectroscopy.

The corresponding copper complexes $[Cu_2(PYP)_2Cl_4]$ (**1**) and $[Cu_4(PYP)_4(ClO_4)_2(H_2O)_2](ClO_4)_2\cdot 2H_2O$ (**2**) were obtained by reacting PYP with $CuCl_2\cdot 2H_2O$ and $Cu(ClO_4)_2\cdot 6H_2O$ in $CH_3OH/CHCl_3$ (2:1) respectively under solvothermal conditions. $[Cu_2(PYP)_2Cl_4]_n$ (**3**) was obtained in the same manner in CH_3OH/H_2O (4:1) (Scheme 2). These complexes were characterized by elemental analysis, ESI-MS and single crystal X-ray diffraction analysis.

2.2. Crystal structure

The molecular structures of the three complexes $[Cu_2(PYP)_2Cl_4]$ (**1**), $[Cu_4(PYP)_4(ClO_4)_2(H_2O)_2](ClO_4)_2\cdot 2H_2O$ (**2**) and $[Cu_2(PYP)_2Cl_4]_n$ (**3**) are depicted in Fig. 1–3. Complexes **1** and **3** have dinuclear structures, whereas complex **2** has a tetranuclear structure. As shown in Fig. 1, each copper(II) in **1** is chelated by one bidentate PYP with the two Cu(II) centers bridged by two μ_2-Cl anions. Each Cu(II) center adopts a distorted square pyramid geometry and is surrounded by three Cl and two N atoms from PYP. Complex **2** in Fig. 2 resembles $[(LCu_2)_2(CO_3)(H_2O)_2(ClO_4)](ClO_4)_3$ (L = bis(tridentate) pyrazolate-based ligand) [33]. Cu(1), Cu(2), Cu(1A) and Cu(1A), Cu(2), Cu(2A) are each spanned by one μ_3-κO,κO′,κO″-bridging perchlorate anion, respectively. The Cu(1) and Cu(1A) centers adopt a distorted tetrahedron geometry and are surrounded by one O atom from the perchlorate anions, one water molecule, and two N atoms from PYP. The distorted tetrahedron geometry of the Cu(2) and Cu(2A) centers are formed by two O atoms from two different μ_3-perchloride anions and two N atoms from PYP. As depicted in Fig. 3, complex **3** has a one-dimensional infinite zigzag chain structure, in which the adjacent two $PYPCuCl_2$ building blocks are bridged by one μ_2-Cl anion and each Cu(II) center adopts a distorted square pyramid geometry completed by one terminal Cl^-, two μ_2-Cl, and two N atoms from PYP. It is worth pointing out that the dimeric structures of **1** and **2** as well as the polymeric structure of **3** can disassociate into mononuclear species in solution, as confirmed by their ESI-MS spectra.

2.3. In vitro cytotoxicity

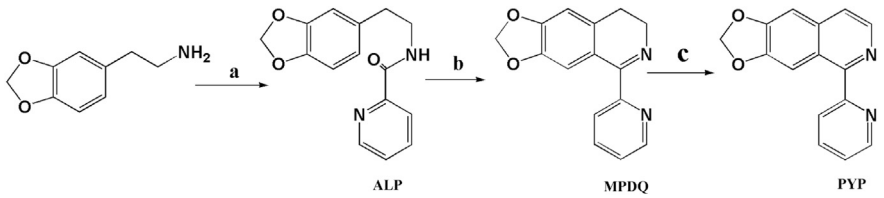
The in vitro cytotoxicity of PYP and complexes **1**–**3** against BEL-7404, SK-OV-3, A549, A375, MGC-803, NCI-H460 and HL-7702 cell lines was investigated with cisplatin as the positive control. As shown in Table 1, the IC_{50} values of complexes **1**–**3** are lower than that of cisplatin. Significantly enhanced activities can be seen for these complexes compared to free PYP and the corresponding copper(II) salts, suggesting the synergistic effect between PYP and copper ions. It should be noted that these copper(II) complexes towards the normal human liver HL-7702 cells displayed lower cytotoxicity than that of them to the tested cancer cells. Thereby, complexes **1**–**3** exhibited a certain extent selectivity to cancer cells. Among these complexes, complex **1** has the highest cytotoxicity against BEL-7404, A549, A375, and NCI-H460, with the IC_{50} values of ranging from 0.31 to 4.29 μM. Complex **3** also exhibits strong cytotoxicity against BEL-7404, A549, MGC-803 and NCI-H460, with the IC_{50} values in the range of 1.32–8.36 μM. Based on their structures and the molecular species in solution (ESI-MS data), it can be concluded that their cytotoxicity originates from the copper ion and the approximately planar benzo[1,3]dioxole moiety of PYP that can intercalate between adjacent base pairs of DNA [12]. Since complex **1** displays the highest cytotoxicity in most cases, it is used as a representative compound in the following studies on the mechanism of cell cytotoxicity against the BEL-7404 cancer cell lines.

2.4. Apoptosis study by flow cytometry

To determine whether the observed cell death induced by the complexes was due to apoptosis or necrosis, the interactions of BEL-7404 cells with the complexes were further investigated using an Annexin V-FITC/propidium iodide assay. As phosphatidylserine (PS) exposure usually precedes loss of plasma membrane integrity in apoptosis, the presence of annexin V+/PI− cells can be considered as an indicator of apoptosis. In the case of complex **1** (Fig. 4), the population of annexin V+/PI− cells (Q4) is 36.8%, which suggests that apoptotic death was induced in BEL-7404 cells.

2.5. Changes in the mitochondrial membrane potential

Mitochondria act as a point of integration for apoptotic signals originating from both extrinsic and intrinsic apoptotic pathways. Mitochondrial dysfunction and the release of apoptogenic factors are critical events in triggering various apoptotic pathways. Loss of mitochondrial membrane potential is an important indicator of cell mitochondrial dysfunction. To evaluate the function of mitochondria in complex **1** induced apoptosis, the changes in mitochondrial membrane potential were measured by the JC-1 probe, which would dissociate from the aggregated form (red fluorescence) to



Scheme 1. Synthesis of PYP ligand. (a) $SOCl_2$, 2-Picolinic acid, K_2CO_3, CH_2Cl_2. (b) $POCl_3$, Toluene, 5%NaOH. (c) Activated MnO_2, Toluene.