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Original article

Copper complexes with phosphonium containing hydrazone ligand: Topoisomerase inhibition and cytotoxicity study

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ABSTRACT

Four new copper(II) complexes containing phosphonium substituted hydrazone (L) with the formulations [CuL]Cl(**3**), [Cu(phen)L]Cl(**4**), [Cu(bpy)L]Cl(**5**), [Cu(dbpy)L]Cl(**6**), (where L = doubly deprotonated hydrazone; phen = 1,10′-phenanthroline; bpy = 2,2′-bipyridine; dbpy = 5,5′-dimethyl-2,2′-bipyridine) have been synthesized. The compounds were characterized by elemental analysis, spectroscopic methods and in the case of crystalline products by X-ray crystallography. The cytotoxicity and topoisomerase I (topo I) inhibition activities of these compounds were studied. It is noteworthy that the addition of N,N-ligands to the copper(II) complex lead to the enhancement in the cytotoxicity of the compounds, especially against human prostate adenocarcinoma cell line (PC-3). Complex **4** exhibits the highest activity against PC-3 with the IC_{50} value of 3.2 μM. The complexes can also inhibit topo I through the binding to DNA and the enzyme.

1. Introduction

Success of cisplatin as an anticancer agent has drawn the attention of many chemists towards the development of new metal based drugs in order to overcome the drawbacks of cisplatin [1–4]. Copper is well known as a bio-essential element for human and its complexes have proved to be excellent for biological applications which include the treatment of cancers [5–8]. Over the last decade, Schiff bases are gaining prominence in medicinal chemistry due to its great chemotherapeutic application [9,10]. It is believed that the type of coordinated ligands and the geometrical orientation of the ligands are crucial factors in promoting the interaction of a given metal complex with DNA.

Recently, Krishnamoorthy and co-workers have shown that copper hydrazones complexes have better potential than other metal complexes in the conversion of DNA from supercoiled form (form I) to the nicked circular form (form II) [11]. Apart from that, there are also numerous copper complexes of hydrazone which possess anticancer properties [12–18]. Therefore, copper hydrazones complexes are one of the important candidates in metal based drugs research. On the other hand, heterocyclic bases play a pivotal role in many biological application and significantly enhanced interaction with DNA [19,20]. Cytotoxic activities of several copper(II) complexes have already been evaluated for their potential antitumour activities on different cell lines [21,22]. Topoisomerases have been identified as important targets in cancer chemotherapy and are among the most widely clinically used anticancer drugs [23–25]. Yet, very few metal complexes have been reported to inhibit topoisomerases in contrast to organic molecules [26,27]. Based on the above findings, we attempt to develop a new series of copper(II) complexes containing 5-(triphenylphosphoniummethyl)-salicylaldehyde benzoylhydrazone] chloride ligand (**2**) and heterocyclic bases such as 1,10′-phenanthroline (phen), 2,2′-bipyridine (bpy) and 5,5′-dimethyl-2,2′-bipyridine (dbpy) as copper based anticancer agents. In order to gain better insight into the mode of action of these potentially cytotoxic compounds, we report herein the first example for the interaction of copper complexes of hydrazone ligand with topoisomerase I.

2. Results and discussion

2.1. Syntheses and characterization

The reaction scheme for the synthesis of copper complexes was presented in Scheme 1. The complexes were highly soluble in DMSO, DMF and methanol. They are non-hygroscopic and stable both in solid and solution phases. The elemental analyses data for

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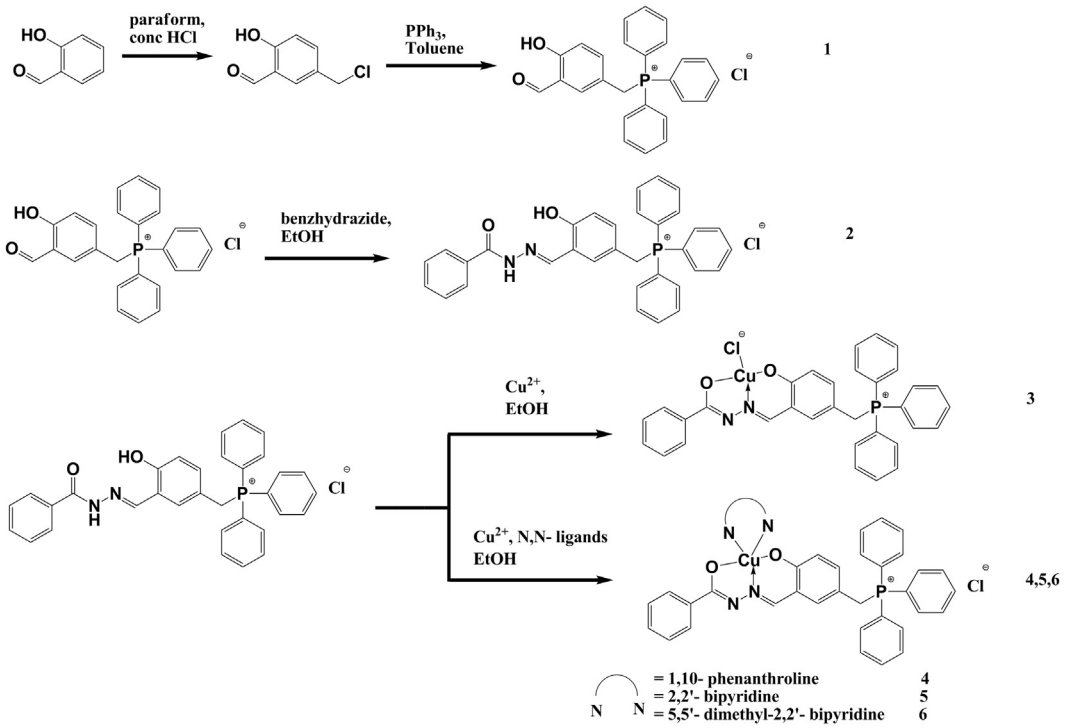

Scheme 1. Reaction scheme and proposed structures.

the Cu(II) complexes are in good agreement with the molecular formulae of the complexes. Compound **1** is white in colour while Schiff base ligand **2** is yellow and all the metal complexes are green.

2.2. Infrared spectra

The IR spectra of the complexes in the region 4000–400 cm^{-1} were analyzed. In comparison with the free ligand, the data gave evidence of coordination of ligand **2** to the copper metal ion *via* phenolate oxygen, azomethine nitrogen and enolate oxygen. In the IR spectrum of **2**, a strong band at 1679 cm^{-1} has disappeared in all the IR spectra of complexes which is assigned to the stretching vibration of carbonyl [ν(C=O)] [19,28]. The infrared spectra of **3**–**6** display IR absorption bands at 1613, 1612, 1612, and 1612 cm^{-1} respectively which are ascribed to the ν(C=N) stretching frequencies of the complexes, whereas for the free ligand the same band was observed at 1619 cm^{-1}. The shift of this band on complexation towards lower wavenumbers indicates the coordination of azomethine nitrogen ν(C=N) to the copper centre [11,18,29]. The appearance of the bands in the 1500–1503 cm^{-1} range in the complexes are due to the asymmetric stretching vibration of the newly formed N=C bond as a result of the enolization of ligand (**2**) [11,30]. In addition, the characteristic ν(N–N) stretching of the free ligand **2** (observed at 1028 cm^{-1}) undergoes a positive shift to a higher wavenumber upon complexation which is attributed to the diminished repulsion between the lone pairs of adjacent nitrogen atoms [31–33]. The formation of complexes **3**–**6** have been confirmed by the presence of bands around ~489 and ~517 cm^{-1} corresponding to ν(Cu–N) and ν(Cu–O) respectively [19,28,30].

2.3. Electronic spectra

The significant electronic absorption bands in the spectra of the ligand **2** and all the complexes recorded in methanol solution are presented in Table 1 and the spectra are shown in Figs. S1 and S2. The bands in the region of 312–328 nm and 228–276 nm of complexes **3**–**6** are due to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the hydrazone ligands and the coordinated diimine ligands [19,29]. The $n \rightarrow \pi^*$ transition, which appears in the region of 289–300 nm in the spectrum of the uncomplexed hydrazone ligand was slightly shifted to a higher wavelength upon complexation. This is an indication of the enolization followed by the deprotonation of the ligand during complexation [30]. The broad bands observed at approximately 400 nm are attributed to the ligand-to-metal-charge transfer (LMCT) transition [11,29,33]. Their broadness may be due to the overlapping of the LMCT transitions of O → Cu and N → Cu. The complexes with N,N donor ligands (**4**–**6**) displayed d–d bands in the 649–655 nm range, which tend to be indicative of a distorted square pyramidal geometry [19] while complex **3** that displays a square planar coordination, absorbs at 636 nm [34].

2.4. ^1H- and ^{13}C- NMR

Additional structural information can be deduced from the ^1H NMR and ^{13}C NMR spectra and relevant chemical shifts are presented in Tables S1 and S2. Since copper(II) complexes are paramagnetic in nature, its NMR spectrum could not be obtained. In the ^1H NMR spectrum of compound **1**, the chemical shift for the aldehydic proton appears at 10.10 ppm. Upon the formation of Schiff base ligand **2**, the aldehydic proton (–CHO) is replaced by azomethine proton (N=CH) which is shifted upfield to 8.46 ppm. The formation of ligand **2** is further corroborated by the presence of –NH proton at 12.24 ppm. The multiplets that appear at the region of 7.62–7.89 ppm are ascribed to the aromatic protons from triphenylphosphine, whereas the aromatic protons of the benzhydrazide appear in the region of 7.49–7.59 ppm and 7.91–7.93 ppm. The sharp doublets signal around 5.00 ppm is assigned to the methylene proton.