Middle-East Journal of Scientific Research 13 (1): 69-73, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.13.1.6413

Synthesis, Characterization and Antitumor Study of N, N'-bis (5-Chloro-2-Hydroxybenzaldehyde) 1, 2-pheylenediimine and its Pt Complex

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Abstract: N,N'-bis(5- Chloro-2- Hydroxybenzaldehyde)1,2-Pheylenediimine abbreviated as (CHBPhD) was synthesized and characterized. This compound used as ligand for preparation of a new Pt complex by reaction with K_2PtCl_4 methanol solution. Characterization of this ligand and its complex was made by microanalyses, FT-IR, ^1H NMR, ^{13}C NMR and UV-Visible spectroscopy and molar conductance measurements. The molar conductance measurements reveal the presence of 1:1 electrolytic nature complexes. These new complexes showed excellent antitumor activity against one kind of cancer cells that is MCF-7 (human breast cancer) cells.

Key words: N,N'-Bis(5-Chloro-2-Hydroxybenzaldehyde)1,2-heylenediimine · Pt Complex · Antitumor Activity · MCF-7 (Human Breast Cancer)

INTRODUCTION

A large number of Schiff bases compounds are often used as ligands in coordination chemistry by considering their metal binding ability. Some Schiff bases were reported to possess antibacterial, antifungal and antitumor activities [1-3]. The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species [4-5]. Several Schiff base complexes have also been shown to inhibit tumor growth [6]. The effect of the presence of various substituents in the phenyl rings of aromatic Schiff bases on their antimicrobial activity has been reported [7]. It was also reported that salicylaldehyde derivatives with halo atoms in the aromatic ring, showed variety of biological activities, like antibacterial activities [3, 4, 8]. Nitrogen-containing ligands such as Schiff bases and their metal complexes played an important role in the development of coordination chemistry. Resulting in an enormous number of publications, ranging from pure synthetic work to physicochemical [9] and biochemically relevant studies of metal complexes [10-14] and found wide range of applications.

Various antitumor Pt complexes were prepared, with an aim to synthesize the 2nd generation Pt complexes with high and specific antitumor activity without or least toxicity. Cisplatin (cis-diamminedichloroplatinum (II)) is still one of the most widely used anticancer drugs in the treatment of various tumors such as testicular, ovarian, head and neck cancers [15-17]. Its antitumor activity is related to the kinetics of the ligand replacement reaction. A very good review on the chemistry of cisplatin in aqueous solution was published [18]. As a routine chemotherapeutic agent for a broad range of solid malignancies, cisplatin functions by cross-linking DNA strands through coordination of nucleic acid bases, which can subsequently induce apoptosis in cancer cells [19] and Mancin *et al*. [20] To determine the antitumor activities of the drugs, the interaction of the pt complexes and CHBPhD with MCF-7(human breast cancer) was assessed. Here, we report the synthesis and chemical characterization of new nitrogen-containing ligand platinum (II) complex.

MATERIALS AND METHODS

Experimental Details: 1, 2-Ethylendiimine was Merck chemicals (Darmstadt, Germany) and was used without further purification. Organic solvents were reagent grade. The UV-Visible measurements were made on a Camspec model 350 spectrophotometer. The IR spectra were recorded using FT-IR model

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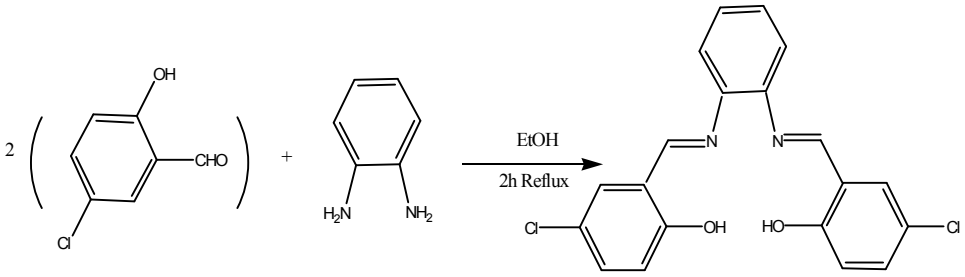

Fig. 1: Synthesis of the CHBPhD ligand.

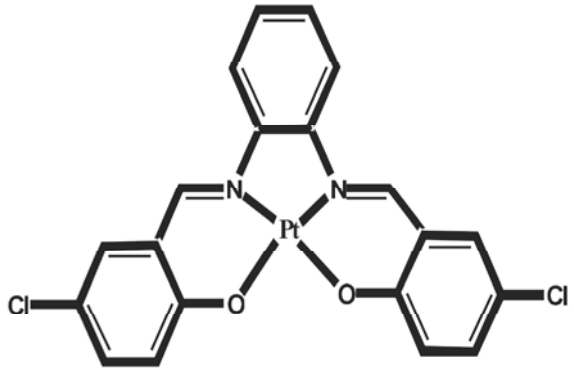

Fig. 2: Synthesis of Metal Complex.

PERKIN-ELMER 843 spectrometer. ^{1}H-NMR and ^{13}C-NMR were recorded on a NMR 500 MHz spectrometer. All the chemical shifts are quoted in ppm using the high-frequency positive convention; ^{1}H and ^{13}C-NMR spectra were referenced to external DMSO. The percent composition of elements was obtained from the Department of Chemistry, Micro analytical Laboratories, Shahid Beheshti University, Tehran.

Cell Culture: MCF-7(human breast cancer) cell line, used for treatment with the drugs, was provided. Human breast cancer cells were grown in an atmosphere containing CO_2, with RPMI-1640 Medium DMEM Modification with L-glutamine and 25 mM HEPES (Sigma-Aldrich Chemie GmbH, Germany) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Carlsbad, Calif, USA), 3.7 g sodium bicarbonate and 500 mg/L ampicillin.

Synthesis of the CHBPhD Ligand: The CHBPhD Schiff base ligand was prepared by refluxing of 5-chloro 2-hydroxybenzaldehyde (4mmole) with 1, 2 ethylenediamine (2 mmol) at ethanol solution and after 2 hours yellow precipitation was formed. The precipitated solid compound was filtered, washed with 50% (v/v) ethanol – water several times to remove any traces of the unreacted starting materials (Figure 1).

Synthesis of Metal Complex: General Method: The 0.3 mmol of methanolic solution of K_2PtCl_4 was added gradually to a stirred solution of the above ligands. The reaction mixture was further stirred for 12 hours to ensure the completion and precipitation of formed a green complex. The precipitated solid complex was filtered, washed with 50% (v/v) ethanol – water several times to remove any traces of the unreacted starting materials. Finally, the complex were washed with diethyl ether and dried in vacuum desiccators over (Figure 2).

RESULTS AND DISCUSSION

Preparation for Ligand, CHBPhD and Pt (II) Complex: The reaction of Pt (II) salt with the ligand, CHBPhD, results formation of [PtL] complex, is quite stable and could be stored without any appreciable change. Complex was characterized by several techniques using elemental analyze (C, H, N), FT-IR, electronic spectra. The elemental analysis data suggest the stoichiometry to be 1:1 [M: L] ratio formation. The molar conductance measurements confirmed the presence of 1:1 and 1:2 electrolytic nature complexes. The complex does not have sharp melting points and decomposed above 200^{0}C. It is insoluble in common organic solvents, such as methanol, chloroform, water or acetonitrile. Its elemental analysis is in accordance with its proposed formula. The spectral data of the complexes have good relationship with the literature data.

Analysis of CHBPhD Ligand and its Pt Complex

Infrared spectra: The IR data of the Schiff base ligands and their pt(II) complexes are listed in Table 1. The IR spectrum of the complex are compared with the free ligands in order to determine the coordination sites that may involved in chelation. The position and the intensities of these peaks are expected to be changed expected chelation.

Table 1: The IR Data of the Schiff Base Ligands and Their Pt(II) Complexes are Listed.

Compound	□(Pt-O)	□(C=C)	□(C=N)	□(Pt-N)
CHBPhD	-	s1564	s1612	-
[Pt(CHBPhD)]	444 vm,464 vm	s 1507	s1608	vw 553

Weak: w¡strong: s ¡very weak: vw ¡medium: M, broa: br

Table 2: UV-Visible Spectroscopic Studies Were Carried Out for the Free Ligand and Complex in DMSO are listed.

	λ_{max}(nm)		
COMPONDE	Π→Π*	n→Π*	d→d Transition
CHBPhD	265	345	-
[Pt(CHBPhD)]	325	452	711

Table 3: 72 hours IC50 and Values (μM) Obtained for Three Compounds.

Compound	IC_{50} on MCF-7 cell line (µg/ml)
Pt(CHBPhD)	$10.66^{(50)}$

^{1}H-NMR spectroscopic studies were carried out for the free ligand and complex in DMSO with use of TMS as the iner standard. ^{1}H-NMR spectra of the ligands, displayed signals corresponding to the various protons. The cyclic aromatice proton appeared as a singlet at 6.9 - 7.7 The OH protons appeared as a singlet at 13.4, in ^{1}H-NMR spectrum of the complex the OH signal was disappeared.

Elemental analysis: Found: C 35.98 %, H 2.25 %, N 5.24 %. Calcd. For?Pt (CHBPhD), $C_{16}H_{12}N_2O_2Cl_2Pt$ (M_r = 530.25): C 36.21 %, H 2.27 %, N 5.28 %. UV-visible spectroscopic studies were carried out for the free ligand and complex in DMSO are listed in Table 2. As seen new signal appeared at 711 nm in complex.

***In vitro* Activities:** CHBPhD ligand and Pt complex were assayed for cytotoxicity *in vitro* against MCF-7(human breast cancer) cell. The cell line was provided by the Pasteur Institute in Iran. The procedure for cytotoxicity studies was similar to that reported earlier [21]. Briefly, in order to calculate the concentration of each drug that produces a 50% inhibition of cell growth (IC_{50}), 190mL of cell suspension 4×10^5 cell/mL) was exposed to various concentrations of ligand and complex dissolved in sterile DMSO. The final concentration of DMSO in the growth medium was 2% (v/v) or lower, concentrations without effect on cell replication [22-23] after the incubation periods 72 hours for all cell lines, the cell concentrations were determined both in control and in drug-treated cultures. All experiments were repeated for six times.

Cytotoxicity Assays *in vitro*: CHBPhD ligand and Pt complex has been tested against one human cancer cell line: The MCF-7(human breast cancer) cell. The IC_{50} cytotoxicity value of the complex was compared to those found for the starting organic bases as well as for some of the anticancer agents used nowadays, that are cisplatin and oxoplatin compounds [24]. The general method used for testing of antitumor properties of these compounds is the standard testing method that has been previously described in greater detail. After incubation lasting for 12 hours at 37°C in a pre 5% CO_2 atmosphere and 100% humidity, the tested compounds in the concentration ranges of 0.1–250iM for CHBPhD. The incubation lasted for 72 hours and at *the* end of this period IC_{50} of the dead cells and live cells were measured by trypan blue. The mechanism by which these complexes act as antitumor agents is apoptosis. IC_{50} values that are the compounds concentrations lethal for 50% of the tumor cells were determined both in control and in compounds concentrations lethal for both in compounds-treated cultures. The compounds were first dissolved in DMSO and then filtrated. The corresponding 50% inhibitory dose (IC50) values are shown in Table 3.

CONCLUSION

It is clear from the above discussion that Pt(II) complex and CHBPhD ligand offer a new outlook for chemotherapy. The result of antitumor activities show that the metal complex exhibit antitumor property and it is important to note that it shows enhanced inhibitory activity compared to the parent ligand.

The mechanism by which these complex act as antitumor agents is apoptosis. It has also been proposed that concentration plays a vital role in increasing the degree of inhabitation.

ACKNOWLEDGEMENT

We gratefully acknowledge the financial support from the Imam Khomeini International University and technical supports that are provided Shahid Beheshti Medical University Tehran.

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