HL
http://heteroletters.org
ISSN: 2231 – 3087(print) / 2230 – 9632 (Online)
Vol. 1: (2), 2011, 106-111

SYNTHESIS, CHARACTERIZATION AND ANTITUMOR STUDY OF N, N-BIS (5- BROMO-2- HYDROXYBENZALDEHYDE)-1, 2-ETHYLENDIIMINE AND ITS PT COMPLEX

Mohammad Reza Rezakhani[a], Shahriar Ghammamy[a,*], Gholamreza Rezaei Behbahani[a], Hajar Sahebalzamani[b], Zahra Shokri Aghbolagh[a], Mahbobeh Tanhayi[a]

[a]Department of Chemistry, Faculty of Science, Imam Khomeini International University, Ghazvin, Iran
[b]Department of Chemistry, Young Researcher Club, Ardebil Branch, Ardebil, Iran
**E-mail: shghamami@ikiu.ac.ir or shghamami@yahoo.com*
Tel: 0098-281-3780040 or 0098-9123162933
Fax: (0098) 281-3780040

Abstract

N,N′-bis(5-Bromo-2-Hydroxybenzaldehyde)1,2-ethylendiimine abbreviated as BHBED 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, ^{1}H NMR, ^{13}C NMR, and UV-Visible spectroscopy and molar conductance measurements. The molar conductance measurements reveal the presence of 1:1 electrolytic nature complex. These new complex showed excellent antitumor activity against one kind of cancer cells that is MCF-7 (human breast cancer) cells.

Keywords: N, N′-bis(5-Bromo-2-Hydroxybenzaldehyde)-1,2-ethylendiimine, 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 [1,2]. Due to their multiple implications, the transition metal complexes with Schiff bases, as ligands, are of paramount scientific interest. Schiff base complexes have been used as drugs. Moreover, it is well known that some drug activities, when administered as metal complexes, are being increased [3]. Several schiff base complexes have also been shown to inhibit tumor growth [4]. The effect of the presence of various substituents in the phenyl rings of aromatic Schiff bases on their antimicrobial activity has been reported [5]. It was also reported that salicylaldehyde derivatives with halo atoms in the aromatic ring, showed variety of biological activities, like antibacterial activities [2,6]. 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 [7] and biochemically relevant studies of metal complexes [8-12] and found wide range of applications.
Pt complexes have usually high antitumor activity against leukemia L1210. 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 [1–6]. However, its severe side effects and acquired cross-resistance restrict its clinical use, and therefore, many platinum complexes have been designed and synthesized to overcome such disadvantages of cisplatin [7-15]. 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 [15]. The Pt–OH_2 can then be displaced by nitrogen donor atoms of the nucleobases of DNA. To determine the antitumor activities of the drugs, the interaction of the pt complexes and BHBED with MCF-7(human breast cancer) was assessed. Here, we report the synthesis and chemical characterization of new nitrogen-containing ligand platinum (II) complex.

EXPERIMENTAL SECTION

Materials and Measurements

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 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 gr sodium bicarbonate, and 500 mg/L ampicillin.

Synthesis of the BHBED Ligand

The BHBED Schiff base ligand was prepared by refluxing of 5-bromo 2- hydroxybenzaldehyde (4mole) 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, BHBED, and Pt(II) Complex

The reaction of Pt(II) salt with the ligand, BHBED, 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 complex. The complex does not have sharp melting points and decomposed above 200^0C. 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 BHBED Ligand and its Pt Complex

Infrared spectra:

The IR data of the Schiff base ligands and their pt(II) complex 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.

^{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 ligand, displayed signals corresponding to the various protons. The cyclic aromatice proton appeared as a singlet at 6.7-7.3. The OH protons appeared as a singlet 20 at in ^{1}H-NMR spectrum of the complex the OH signal was disappeared.

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 658 nm in complex.

In Vitro Activities

BHBED 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 [16]. Briefly, in order to calculate the concentration of each drug that produces a 50% inhibition of cell growth (IC_{50}), 190 mL 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 [17,18] 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

BHBED 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 [19].

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 at37 °C in a pre 5% CO_2 atmosphere and 100% humidity, the tested compounds in the concentration ranges of 0.1–250 μM for BHBED. The incubation lasted for 72 hours and at the end of this period IC_{50} and IC_{90} 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} and IC_{90} values that are the compounds concentrations lethal for 90% and 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% and 90% inhibitory dose (IC_{50} and IC_{90}) values are shown in Table3.

CONCLUSION

It is clear from the above discussion that Pt(II) complex and BHBED 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.

ACKNOWLEDGEMENTS

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

REFERENCES

[1] A.S. Gaballa, M.S. Asker, A.S. Barakat, S.M. Teleb, Spectrochim Acta 67, 114 (2007).
[2] (a) L. Shi, H.M. Ge, S.H. Tan, H.Q. Li, Y.C. Song, H.L. Zhu, R.X. Tan, Eur. J. Med. Chem. 42, 558 (2007).
(b) J. Lv, T. Liu, S. Cai, X. Wang, L. Lu, Y. Wang, J. Inorg. Biochem. 100, 1888 (2006).
[3] D.N. Akbayeva, L. Gonsalvi, W. Oberhauser, M. Peruzzini, F. Vizza, P. Bruggeller, A. Romerosa, G. Sava, A. Bergamo, Chem. Commun. 264 (2003).
[4] F.B. Dwyer, E. Mayhew, E.M.F. Roe, A.Br. Shulmon, J. Cancer. 19, 195 (1965).
[5] H.S. Hothi, A. Makkar, J.R. Sharma, M.R. Manrao, Eur. J. Med. Chem. 41, 253 (2006).
[6] L.C. Fenton, J.H. Brewer, Science 105, 409 (1947).
[7] X.F. Luo, X. Hu, X.Y. Zhao, S.H. Goh, X.D. Li, Polymer 44, 5285 (2003).
[8] A.S.N. Murthy, A.R. Reddy, Journal of Chemical Sciences 90, 519 (1981).
[9] V. Razakantoanina, N.K.P. Phung, G. Jaureguiberry, Parasitology Research 86, 665 (2000).
[10] R.E. Royer, L.M. Deck, T. Vander Jagt, Journal of Medicinal Chemistry 38, 2427 (1995).
[11] M.R. Flack, R.G. Pyle, N.M. Mullen, Journal of Clinical Endocrinology & Metabolism 76, 1019 (1993).
[12] R. Baumgrass, M. Weiwad, F. Erdmann, Journal of Biological Chemistry 276, 47914 (2001).
[13] B. Lippert, Chemistry and Biochemistry of a Leading Anticancer Drug, Wiley- VCH, Weinheim, 1999.
[14] S.J. Berners-Price, T. G. Appleton, Platinum-based Drugs in Cancer Therapy, Humana Press Inc, 3 (2000).
[15] M.J. Cleare, P.C. Hydes, B.W. Malerbi, D.M. Watkins, Biochimie 60, 835 (1978).

[16] H. Lin, S. Zhu, H. Sun, Y. Chen, Journal of Inorganic Biochemistry 70, 219 (1998).
[17] J. Ishida, H.K. Wang, K.F. Bastow, C.Q. Hu, K.H. Lee, Bioorganic & Medicinal Chemistry Letters 9, 3319 (1999).
[18] J.K. Son, L.X. Zhao, A. Basnet, European Journal of Medicinal Chemistry 43, 675 (2008).
[19] Y.S. Kim, R. Song, H.C. Chung, M.J. Jun, Y.S. Sohn, Journal of Inorganic Biochemistry 98, 98 (2004).

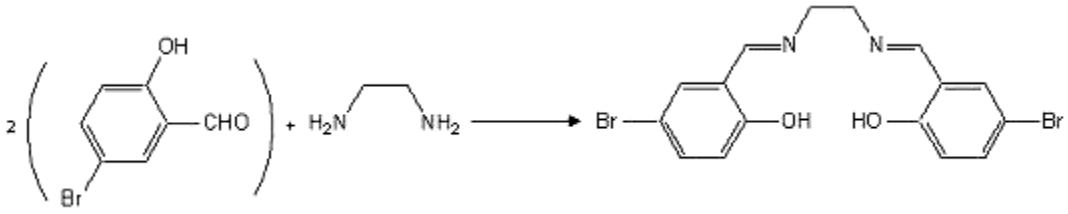

Figure1. Synthesis of the BHBED ligand

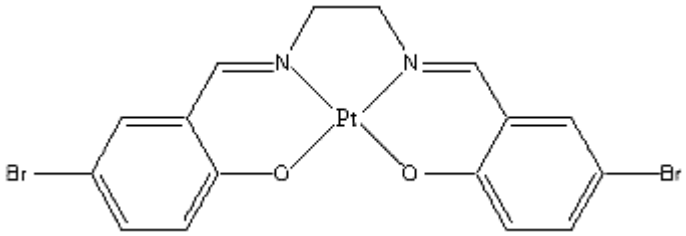

Figure 2. Chemical structure of Metal Complex

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

Compound	ν(Pt-O)	ν(C=C)	ν(C=N)	ν(Pt-N)
BHBED	-	1564s	1632s	-
[Pt(BHBED)]	414 vm,418 vm	1581 s	1638s	563 vw

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.

COMPONDE	λmax		
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	d→d Transition
BHBED	267	325	-
[Pt(BHBED)]	34 0	425	655

TABLE 3. 72 hour IC50 and Values (μM) Obtained for Three Compounds

Compound	IC_{50} on MCF-7 cell line (μg/ml)
Pt(BHBED)	$41.01^{(50)}$