

Synthesis, Characterization and Thermal Studies of A N, N'-Bis(2-Hydroxy-Alpha-Methyl Benzylidene)-P-Benzyl diamineuranyl (VI) Nitrate[UO₂(HMPBD)]²⁺

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Abstract: N, N'-bis(2-hydroxy-alpha-methyl benzylidene)-p-benzyl diamine abbreviated as HMPBD was synthesized and characterized. N, N'-bis(2-hydroxy-alpha-methyl benzylidene)-p-benzyl diamineuranyl (VI) nitrate prepared by reaction of nitrate salt of UO₂(NO₃)₂·6H₂O with HMPBD. In this research, some of the inorganic complexes of uranyl with N-donor ligands were synthesized. Complexes were characterized by FT-IR and UV, ¹HNMR, ¹³CNMR spectra, TG/DTG measurements and some physical properties. The results of simultaneous TG-DTG-DTA analyses of the complexes show the final degradation product for these complexes are UO₃. The antitumor activity of used ligands and their complexes against a panel of human tumor cell lines (HT29: Human colon adenocarcinoma cell line T47D: human breast adenocarcinoma cell line) were studied and determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay. These data suggest that some of these compounds provide good models for the further design of potent antitumor materials. Also the results show chelation causes drastic change in the biological properties of the ligands and also the metal moiety. So the toxic effects of uranyl can be prevented by using chelating agent and complexation of the potentially multidentate ligands.

Key words: N • N'-Bis(2-Hydroxy-Alpha-Methyl Benzylidene)-P-Benzyl Diamineuranyl (VI) Nitrate • Synthesis • Thermal Analysis • FT-IR And UV-Visible Spectroscopy • Schiff Bases • Anticancer Activity

INTRODUCTION

There is an inverse proportionality between industrial importance and scientific interest in the case of uranyl compounds. Many efforts have been done on preparation of new uranyl compounds. The coordination chemistry of transition metals with ligands from the uranyl family has been of interest due to different bonding modes shown by these ligands with both electron rich and electron poor metal. In principle, the central transition metal atoms of different soft and hard Lewis acidity usually need to be satisfied in the most suitable fashion. Schiff base metal complexes have been widely studied because they have industrial, antifungal, antibacterial, anticancer and herbicidal applications.

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 [1] and biochemically relevant studies of metal complexes [2-6] and found wide range of applications. Other kinds of nitrogen-containing ligands are well-known pyrimidine systems such as purine analogues that exhibit a wide range of biological activities. Fused pyrimidine compounds are valued not only for their rich and varied chemistry, but also for many important biological properties. Among them, the furopyrimidine ring system, because of a formal isoelectronic relationship with purine, is of special biological interest. It has numerous pharmacological and agrochemical applications, namely, antimalarials, antifolates and antiviral, as well as potential radiation protection agents. Recently, some furopyrimidines were shown to be potent ascular endothelial growth factor receptor 2 (VEGFR2) and epidermal growth factor receptor (EGFR) inhibitors [7]. Because of the importance of furo (2,3-d) pyrimidine derivatives, several methodologies for synthesizing them have already been developed.

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However, many of the synthetic protocols reported so far prolonged reaction times, harsh reaction suffer from disadvantages, such as relying on multistep reactions, needing anhydrous conditions, low yields, use of metal-containing reagents and special instruments or starting materials. Therefore, the development of new and efficient methods for the preparation of furo (2,3-d) pyrimidine derivatives is still strongly desirable [7]. Pyrimidines represent a very interesting class of compounds because of their wide applications in pharmaceutical, phytosanitary, analytical and industrial aspects, for example, as antibacterial, fungicide [8], antihelmintics, antitubercular, anti-HIV, antidegenerative and hypothermic activities [8] and herbicides, and have biological activities [9-13]. It has long been known that metal ions involve in biological processes of life and have been subject of interest. The modes of action of these metal ions are often complex but are believed to involve bonding to the heteroatom of the heterocyclic residues of biological molecules, that is, proteins, enzymes, nucleic acids and so forth [14]. From these points of view, it is interesting to study different types of transition metal complexes of these biologically active ligands. In this paper, the synthesis characterization and antitumor properties of a number of the ligands and uranyl complexes have been studied. In this work, we report the synthesis and structural studies of the ligand and complex isolated from the reactions of: N, N'-bis(2-hydroxy-alpha-methyl benzylidene)-p-benzyl diamineUranyl (VI) nitrate.

MATERIAL AND METHODS

Solvents were purified by standard methods. All reagents were supplied by Merck and were used without further purification. Melting point was determined in an Electro thermal 9200. The FT-IR spectra were recorded in the range 400-4000 cm^{-1} by KBr disk using a Bruker Tensor 27 M 420 FT-IR spectrophotometer. The UV-Vis spectra in CH_3CN were recorded with a WPA bio Wave S2 100 spectrophotometer. Thermo gravimetric analyses were done on a Perkin Elmer TGA/DTA lab system 1 (Technology by SII) in nitrogen atmosphere with a heating rate of $20^\circ\text{C}/\text{min}$ from $35-700^\circ\text{C}$. ^1H and ^{13}C -NMR spectra were measured on a BRUKER DRX-500 AVANCE spectrometer at 500 MHz.

Cell Culture: The human tumor cell line (H29: human colon adenocarcinoma cell line, T47D: human breast adenocarcinoma cell line, used for treatment with the drug

was provided. H29 and T47D Cell were grown at 37°C in an atmosphere containing 5% CO_2 , wet 95% with RPMI-1640 Medium HEPES Modification with 2mM L-glutamine and 25 mM HEPES (Sigma-Aldrich Chemie GmbH, Germany) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Carlsbad, Calif, USA), 20g/L sodium bicarbonate and 500 mg/L (100unit/ml) ampicillin, streptomycin 100 $\mu\text{g}/\text{ml}$.

Cytotoxicity Studies: HMPBD ligand and $[\text{UO}_2(\text{HMPBD})]^{2+}$ complex are two compounds which were assayed for cytotoxicity in Cytotoxicity studies against (HT29: Haman colon adenocarcinoma) cells and T47D: human breast adenocarcinoma cell line) cells. The two cell lines were provided by the Pasteur Institute in Iran. The procedure for cytotoxicity studies was similar to that reported earlier [15]. 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/ cm^3) was exposed to various concentrations of ligand and complexes 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. After the incubation period's 72 hours for all cell lines, the cell concentrations were determined both in control and in drug-treated cultures. All experiments were done for six times.

Synthesis of the $[\text{UO}_2(\text{HMPBD})]^{2+}$: For synthesis of the $[\text{UO}_2(\text{HMPBD})]^{2+}$ to a magnetically stirred of ligand (0.47g, 1.4mmol) in acetonitrile(10ml) was added to uranyl (VI) nitrate (0.68g, 1.4mmol) at room temperature. The reaction mixture was further stirred for 3 hours to ensure the completion and precipitation of the formed complex. The precipitated solid complex was filtered and washed several times with diethyl ether to remove any traces of the unreacted starting materials. Yield, 80%. Anal. Calcd of $[\text{UO}_2(\text{HMPBD})]^{2+}$; C; 43.01, H; 3.25, N; 4.55; found: C; 43.68, H. 3.68, N; 4.98. Mp: 250°C . ^1H NMR (DMSO): 7.4-7.8 (CH phenol), 2.5 (CH₃), 6.9 (CH benzyl), FT-IR (KBr, cm^{-1}): 1305 s (μ C-N), 1588 s (μ C=N), 2935 br (μ OH), 580 m (μ U-O), 422 w (μ U-N), 920s (μ O=U=O), UV-vis (DMSO): λ_{max} 260nm(ϵ 30000), 310nm(ϵ 9000), 400nm(ϵ 2000) (Figure 1-9). $[\text{UO}_2(\text{HMPBD})]^{2+}$ is soluble in acetone, DMF and DMSO and insoluble in diethyl ether, water, Acetonitrile, methanol and hexane and little soluble in chloroform, ethanol and dichloro methane. Figure 10, 11 shows Chemical structures of HMPBD and $[\text{UO}_2(\text{HMPBD})]^{2+}$.

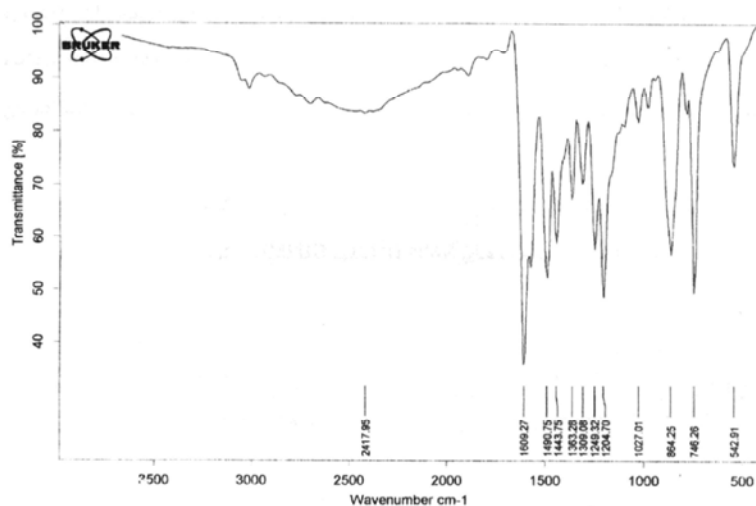


Fig. 1: FTIR spectrum of HMPBD (KBr Disk)

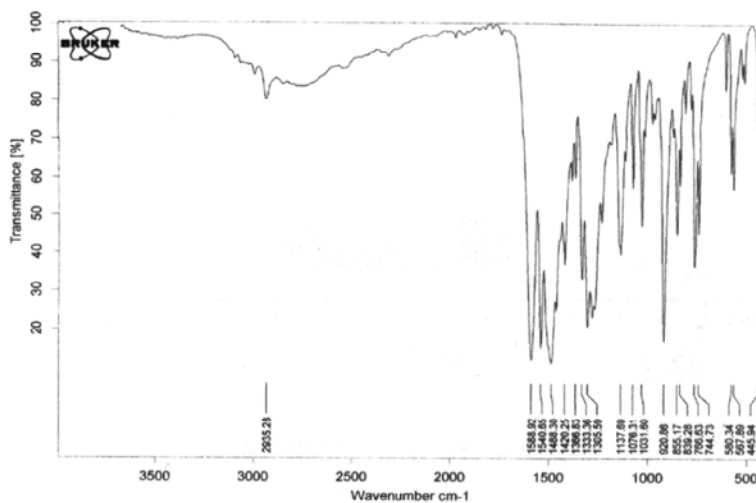


Fig. 2: FTIR spectrum of $[UO_2 (HMPBD)]^{2+}$ (KBr Disk)

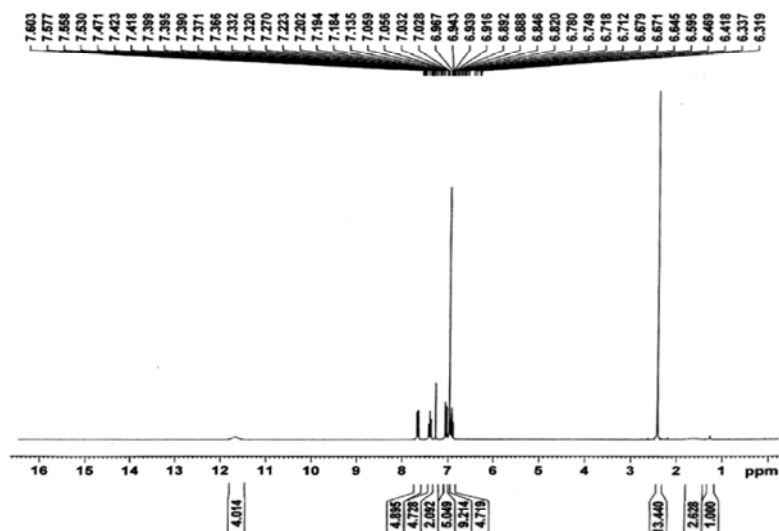


Fig. 3: 1H -NMR spectrum of HMPBD

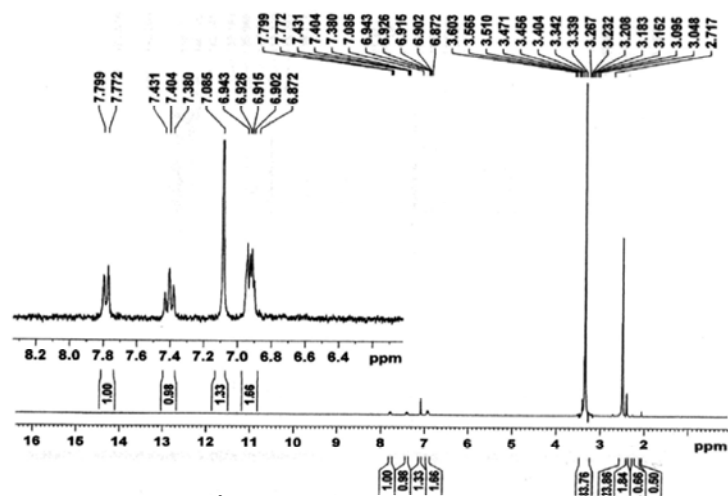


Fig. 4: ^1H -NMR spectrum of $[\text{UO}_2(\text{HMPBD})]^{2+}$

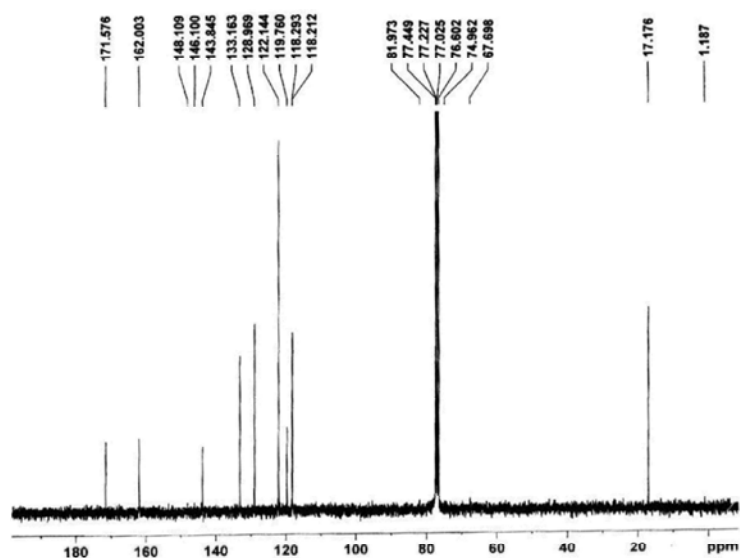


Fig. 5: ^{13}C -NMR spectrum of HMPBD

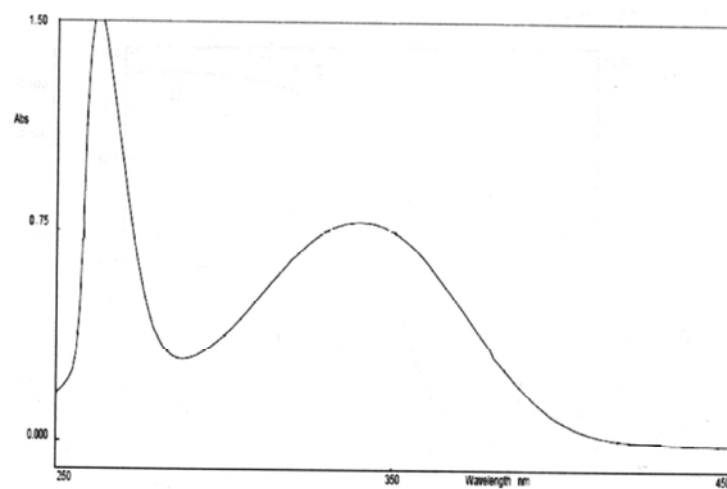


Fig. 6: UV/ Vis spectrum of HMPBD(DMSO, $5 \times 10^{-4} \text{ M}$)

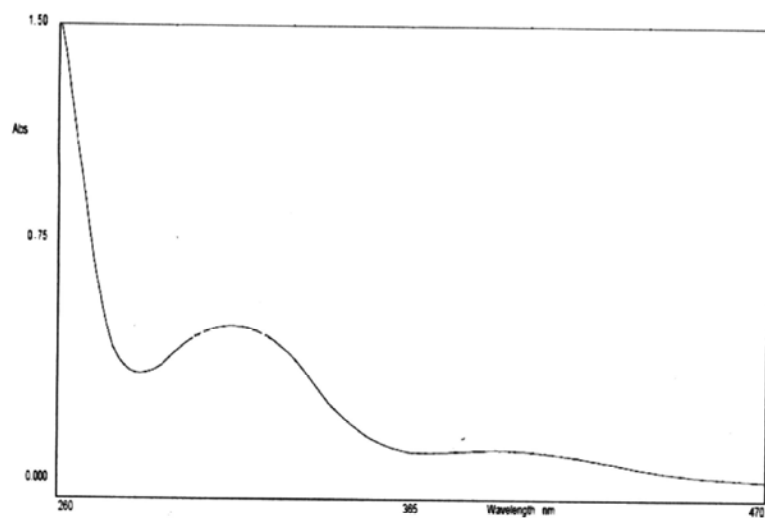


Fig. 7: UV/ Vis spectrum of $[\text{UO}_2(\text{HMPBD})]^{2+}$ (DMSO, 5×10^{-4} M)

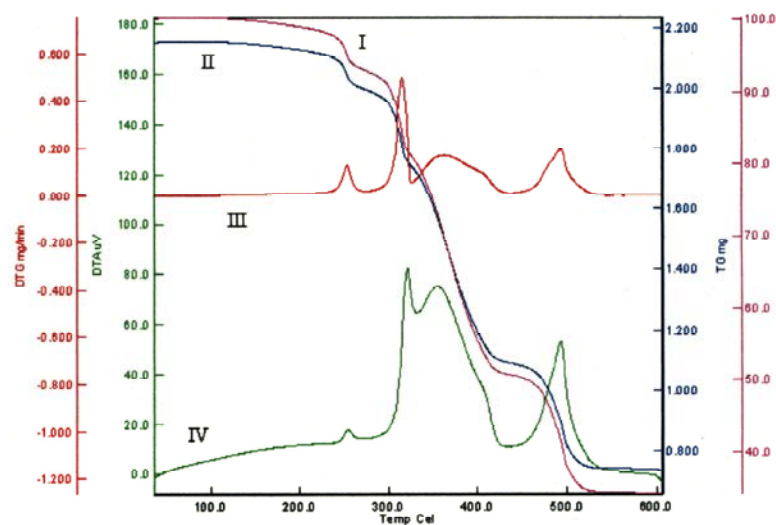


Fig. 8: Thermal analysis data of $[\text{UO}_2(\text{HMPBD})]^{2+}$

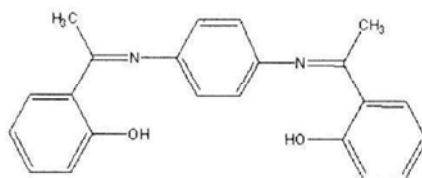


Fig. 9: Chemical structure of HMPBD

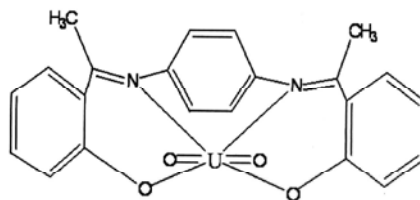


Fig. 10: Chemical structure of $[\text{UO}_2(\text{HMPBD})]^{2+}$



Fig. 11: Morphology cells (HT-29) containing $[\text{UO}_2(\text{HMPBD})]^{2+}$ (0.001M)

Analysis of HMPBD Ligand: Anal: %69. Calcd of $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_2$; C; 76.76, H; 5.81, N; 8.13; found: C; 80.12, H. 6.1, N; 8.51. Mp 233-235 °C, ^1H NMR (DMSO): 7.2-7.6 (CH phenol), 2.4 (CH₃), 6.9 (CH benzyl), 11.7 (OH), FT-IR (KBr, cm^{-1}): 1204s (μ C-N), 1609s (μ C=N), 2417br (μ OH), UV-vis (DMSO): λ_{max} 260nm (ϵ 30000), 340nm (ϵ 15000). HMPBD is soluble in acetonitrile, DMF, DMSO, dichloro methane and chloroform and insoluble in acetone, water, methanol, ethanol, diethyl ether and hexane.

RESULTS

Preparation of Ligand and Complex: The reaction of uranyl nitrate with the ligand in acetonitrile solvent result the formation of $[\text{UO}_2\text{L}]$ in that $\text{L}=\text{HMPBD}$ in the molar ratio 1:1 (metal: ligand). Compounds are quite stable and could be stored without any appreciable changes for long time. Compounds were characterized by several techniques using FT-IR, UV-Visible and NMR spectra. Thermal analysis was studied for these compounds. The $[\text{UO}_2(\text{HMPBD})]^{2+}$ has 250 °C melting points respectively. It is soluble in acetone, DMF and DMSO and insoluble in diethyl ether, water, Acetonitrile, methanol and hexane and little soluble in chloroform, ethanol and dichloro methane. The spectral data of the complexes have good relationship with the literature data.

Cytotoxicity Assays *In vitro*: HMPBD and $[\text{UO}_2(\text{HMPBD})]^{2+}$ are compounds which were assayed for cytotoxicity *in vitro* against (HT29) cells and T47D) cells. The two cell lines were provided by the Pasteur Institute in Iran. The procedure for cytotoxicity studies was similar

to that reported earlier. The concentration ranged from 0.01 to 0.001M for HMPBD and complex in DMSO were prepared. After preincubation lasting for 12 hours at 37 °C in 5% CO_2 atmosphere and 95% humidity the tested compounds. The color intensity was measured by Eliza instrument at 570 and 630 nm. The mechanism by which this complex acts as antitumor agents is apoptosis from the ligand. The life percentage of cell (HT-29) of HMPBD in 0.01 and 0.001 is 16.7 and 36.9, respectively. The life percentage of cell (742) of HMPBD in 0.01 and 0.001 is 11.8 and 29.4, respectively. The life percentage of cell (HT-29) of $[\text{UO}_2(\text{HMPBD})]^{2+}$ in 0.01 and 0.001 is 42.7 and 38.9, respectively. The life percentage of cell (742) of $[\text{UO}_2(\text{HMPBD})]^{2+}$ in 0.01 and 0.001 is 24.1 and 6.1 respectively. (Figure 12)

Thermo Gravimetric Analyses: The thermal properties of these compounds were investigated by thermo grams (TG, DTG and DTA). Figure (8) shows TGA and DTA curves for $[\text{UO}_2(\text{HMPBD})]^{2+}$. In the temperature range 300-420°C, 39.5% weight losing was observed which was related to the loss of most parts of compound. In the temperature range from 480-530°C, 16.3% weight reduction was found, which was related to the loss of a part of compound.

DISCUSSION

In this research, some of the inorganic complexes of uranyl with N-donor ligands were synthesized. Complexes were characterized by FT-IR and UV, ^1H NMR, ^{13}C NMR spectra, TG/DTG measurements and some physical properties. The results of simultaneous TG-DTG-DTA

analyses of the complexes show the final degradation product for these complexes are UO_3 . The antitumor activity of used ligands and their complexes against a panel of human tumor cell lines (HT29: Haman colon adenocarcinoma cell line T47D: human breast adenocarcinoma cell line) were studied and determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay.

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