Abstract—In this research, some of the inorganic complexes of uranyl with N-donor ligands were synthesized. Complexes were characterized by FT-IR and UV, $^1$HNMR, $^{13}$CNMR 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$_2$. 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. 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.

Keywords—Transition Metal, Uranyl, Complexes, Schiff bases, Anticancer Activity.

I. INTRODUCTION

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 isoionic relationship with purine, is of special biological interest. It has numerous pharmacological and agrochemical applications, namely, antimalarials, antifolates, and antivirus, as well as potential radiation protection agents. Recently, some furopyrimidines were shown to be potent ascorul endothelial growth factor receptor 2 (VEGFR2) and epidermal growth factor receptor (EGFR) inhibitors. Because of the importance of furo (2,3-d) pyrimidine derivatives, several methodologies for synthesizing them have already been developed. 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 [9], and have biological activities [10–14]. 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 [15]. 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.

II. MATERIAL AND METHODS

A. Chemical and Reagents

Uranyl (VI) nitrate UO$_3$(NO$_3$)$_2$,6H$_2$O, hydrazine hydrate, Carbon disulfide, 3-methyl 4-amino 5-mercaptu 1,2,4 tri azole thio carbohydrazide, chloroform, acetic acid, para-hydroxy benzene aldehydes were Merck chemicals (Darmstadt, Merck, Germany) and were used without further purification. Organic solvents were reagent Grade. Electronic spectra were recorded by Camsp UV-Visible spectrophotometer model Shimadzu 2100(Wpa bio Wave S2 100). The IR spectra were recorded using FT-IR Bruker Tensor 27 spectrometer (model420). $^1$H-
NMR and $^{13}$C-NMR were recorded on a Bruker AVANCE DRX 500 spectrometer (in DMSO, acetone, CDCl$_3$ solvents). 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 SiMe$_4$. TGA-DTA analysis were recorded using Perkin-Elmer by thermal program 20°C/min in 400-700 °C thermal rang.

B. 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 were 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), 20gr/lit sodium bicarbonate, and 500 mg/L (100uint/ml) ampicillin, ester peto mysin 100micro gram/ml.

III. EXPERIMENTAL

A. General method for synthesizing of ligands

10 cm$^3$ of distilled water and 50 cm$^3$ hydrazine hydrate mixed and heated. This solution was added gradually to 15 cm$^3$ of carbon disulfide during 1 hour and stirred with magnetic stirrer. The reaction mixture was refluxed for 24 hours then kept for about 2 hours and cooled to the room temperature. Then filtered, washed with distilled water and dried at room temperature. To this thiocarbohydrazid precipitate (10.6 gr) was added 50 mL acetic acid. This mixture was refluxed for 2 hours, and then cooled. The solid was filtered, washed with distilled water and dried at room temperature, yielded a white precipitate. To precipitate product substituted-hydroxy benzene aldehyde was added, in 1:1 molar ratio in chloroform solvent, mixing was continued with magnetic stirrer then product was filtered, washed with ethanol and dried. Two these type ligands were been synthesized. Their name and abbreviation are: 4-hydroxy benzyliden aminov5-methylv2, 4-dihydrov3Hv1,2,4-triazolev3-thione, HBAMDT and 3-nitro benzyliden aminov5-methylv2, 4-dihydrov3Hv1,2,4-triazolev3-thione, NBAMDT.

B. Analysis of HBAMDT Ligand

Anal. Calcd of C$_{10}$H$_{10}$N$_4$O$_2$S; C; %45.62, H; 3.42, N; 26.61; found: C; 46.02, H, 3.56, N; 26.93. Mp 232-234 °C, $^1$HNMR (DMSO): 7-8.8 (CH nitro phenyl), 2.3(CH3) 10.5(NH), 9.2(CH azomethin) FT-IR (KBr, cm$^{-1}$): 1530s, 1094m. UV-vis (DMSO): $\lambda_{max}$ 265nm(ε 32000), 310nm(ε 8000).

C. Analysis of NBAMDT Ligand

Anal. Calcd of C$_{10}$H$_{10}$N$_4$O$_2$S; C; %45.62, H; 3.42, N; 26.61; found: C; 46.02, H, 3.56, N; 26.93. Mp 232-234 °C, $^1$HNMR (DMSO): 7-8.8 (CH nitro phenyl), 2.3(CH3) 10.5(NH), 9.2(CH azomethin) FT-IR (KBr, cm$^{-1}$): 1530s, 1094m. UV-vis (DMSO): $\lambda_{max}$ 265nm(ε 32000), 310nm(ε 8000).

D. Synthesis of the [UO$_2$(HBAMDT)$_2$] Complexes:

A solution of uranyl (VI) nitrate salt dissolved in acetonitrile was added gradually to a stirred acetonitrile solution of the ligand (HBAMDT), in the 1:1 (metal: ligand) molar ratio. 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.

E. Analysis of [UO$_2$(HBAMDT)$_2$]

Yield, 88%. Anal. Calcd of [UO$_2$(HBAMDT)$_2$], C$_{20}$H$_{18}$N$_8$O$_4$S$_2$U; C; %32.60, H; 2.44, N; 15.21; found: C; 32.83, H, 2.59, N; 15.36. Mp 110-112 °C $^1$HNMR (DMSO): 6.9-7.7 (CH phenol), 10.2 (OH), 2.5(CH3)), 8 (CH azomethin) FT-IR (KBr, cm$^{-1}$): 1582s, 741m, 946s, 476w, 627m UV-vis (DMSO): $\lambda_{max}$ 265nm(ε 32000), 425nm(ε 13000) [UO$_2$(HBAMDT)$_2$] is soluble in acetone, DMF and DMSO and insoluble in water, chloroform and methanol.
Fig. 2.: Chemical structure of $[\text{UO}_2(\text{HBMDT})_2]^{2+}$

F. Synthesis of the $[\text{UO}_2(\text{NBAMDT})_2]$ complex

NBAMDT ligand (1.6 gr) was solved in acetonitrile (10 ml), obtained white color solution, and then uranyl nitrate ($\text{UO}_2(\text{NO}_3)_2\cdot6\text{H}_2\text{O}$) (1.5 gr) was solved in acetonitrile (10 ml). Yellow color solution salt was added on the ligand solution and stirring with magnetic stirrer. After addition of salt solution on ligand solution the color is changed to brown. After 3 hours stirring precipitate washed with acetonitrile.

G. Analysis of $[\text{UO}_2(\text{NBAMDT})_2]$ complex

Yield, 91%. Anal. Calcd of $[\text{UO}_2(\text{NBAMDT})_2]$: C, 30.22; H, 2.01; N, 17.63; found: C, 30.95; H, 2.12; N, 16.78. Mp 223-225 °C, $^1\text{HNMR}$ (DMSO): 7.9-8.7 (CH nitrophenil), 2.3 (CH$_3$), 9.3 (CH azomethin). FT-IR (KBr, cm$^{-1}$): 1503 s, 751 m, 944 s, 490 w, 626 m. UV-vis (DMSO): $\lambda_{\text{max}}$ 260 nm ($\varepsilon$ 28000), 300 nm ($\varepsilon$ 8000), 380 nm ($\varepsilon$ 3000). $[\text{UO}_2(\text{NBAMDT})_2]$ is soluble in DMSO and dichloro methane and insoluble in water, acetonitrile, methanol, hexane and chloroform.

IV. CYTOTOXICITY STUDIES

HBMDT and NBAMDT ligands and $[\text{UO}_2(\text{HBMDT})_2]$, $[\text{UO}_2(\text{NBAMDT})_2]$ complexes are three compounds which were assayed for cytotoxicity in vitro 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 [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 $4\times10^5$ 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. Fig. 3. shows the morphology of HT-29 cells after 72 hours in contact with $[\text{UO}_2(\text{HBMDT})_2]$ in (0.001M concentration).

V. RESULT AND DISCUSSION

A. Preparation of Ligands and complexes

The reaction of uranyl nitrate with the ligands in acetonitrile solvent resulted the formation of $[\text{UL}_2]$ in that L=HBMDT, NBMDT in the molar ratio 1:2 (metal: ligand). All complexes are quite stable and could be stored without any appreciable changes for long time. All complexes were characterized by several techniques using FT-IR, UV-Visible and NMR spectra. Thermal analysis were studied for these compounds. The $[\text{UO}_2(\text{HBMDT})_2]$, $[\text{UO}_2(\text{NBAMDT})_2]$ complexes have 110-112 °C and 223-225 °C melting point respectively. They are insoluble in common organic solvents, such as ethanol, methanol, chloroform however, they are soluble in DMSO. The spectral data of the complexes have good relationship with the literature data.

B. Cytotoxicity Assays In Vitro

(HBMDT) and NBAMDT ligands and $[\text{UO}_2(\text{HBMDT})_2]$ and $[\text{UO}_2(\text{NBAMDT})_2]$ complexes have been tested against two human cancer cell lines: HT29 and T47D. The IC$_{50}$ cytotoxicity values for these complexes were compared to those found for the starting organic bases as well as for some of the anticancer agents used nowadays, that are cisplatin and oxaplatin compounds [14-16]. The general method used for testing on antitumor properties of these compounds is the standard testing method that has been previously described in greater detail. After preincubation lasting for 12 (24) hours at 37 °C in 5% CO$_2$ atmosphere and 95% humidity the tested
compounds in the concentration ranges of 0.1, 0.01, 0.001 M for CDP and two complexes. 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.

**V. CONCLUSION**

It is clear from the above discussion that [UO$_2$(HBMDT)$_2$] and [UO$_2$(NBAMDT)$_2$] complexes (HBMDT) and (NBAMDT) ligand offer a new outlook for chemotherapy. The results of antitumor activity show that the metal complexes exhibit antitumor properties and it is important to note that they show enhanced inhibitory activity compared to the parent ligand. The mechanism by which these complexes act as antitumor agents is apoptosis. It has also been proposed that concentration plays a vital role in increasing the degree of inhabitation.

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