Uranyle Complexes: synthesis, evaluation of biological activity

Shahriar Ghammamy[1]∗, Rozita Hosseinzadeh Khezri, [1] Zahra Amini, [1]Monir Al-sadat Mirrahimi , [1] Masoud Soleimani[2] and Saeid Kaviani[2]

[1]*Department of Chemistry, Faculty of Science, Imam Khomeini International University, 34149-16818 Ghazvin, Iran*

[2]*Department of Hematology, Faculty of Medicine, Tarbiat Modarres University, P.O. Box 14115-318, Tehran, Iran*

Abstract-In this research, some of the inorganic complexes of uranyl with N- donor ligands were synthesized. Complexes were characteriezed by FT-IR and UV spectra, ^{1}HNMR, ^{13}CNMR and some physical properties. The uranyl unit (UO_2) is composed of a center of uranium atom with the charge (+6) and two oxygen atom by forming two U=O double bonds. The structure is linear (O=U=O, 180) and usually stable. So other ligands often coordinate to the U atom in the plane perpendicularly to the O=U=O axis. The antitumor activity of some of ligand and their complexes against a panel of human tumor cell lines (HT29: Haman colon adenocarcinoma cell line T47D: human breast adenocarcinoma cell line) were 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 compounds.

Key words: Inorganic, Uranyl complex-donor ligands, Schiff bases, Anticancer Activity.

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 isoelectronic 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 vascular endothelial growth factor receptor2 (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, industrial aspects for example as antibacterial, fungicide, antihelmintics, antitubercular, anti-HIV, antidegenerative, hypothermic activities [8], 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 heteroatoms 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 lanthanide metal complexes of these biologically active ligands. In this paper, the synthesis characterization, and antitumor properties of a number of the lanthanide metal complexes with one of the above ligands have been studied.

Materials and Methods

Chemicals and Reagents

Uranyl(VI) nitrate $UO_2(NO_3)_2.6H_2O$, hydrazine hydrate, Carbon disulfide, 3-methyl-4-amino-5-mercupto-1,2,4-thri-azole-thiocarbohydrazide, Chloroform, Acetic acid, para-hydroxy benzealdehyde 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 Brukerec Tensor 27 spectrometer (mode l420). ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker AVANCE DRX 500 spectrometer (in DMSO, acetone, $CDCl_3$ solvent). 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 SiMe4. (TG-DTA analysis were recorded using Perkin Elmer by thermal program 20°C/min in 400-700 °C thermal rang.

Cell Culture

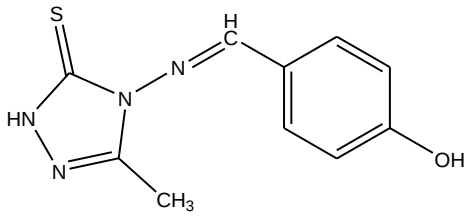
The human tumor cell line H29: human colon adenocarcinoma cell line, T47D: humanbreast adenocarcinoma cell line, used for treatment with the drug were provided. H29 and T47D Cell were grown at 37°C in an atmosphere containing 5٪ CO2, wet 95٪ with RPMI-1640 Medium HEPES. Modification with 2Mm L-glutamine 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.

Experimental

Synthesis of the HBAMDT Ligand

10 cc distilled water and 50 cc hydrazine hydrate 100٪ mixed and heated. This solution was added gradually to 15 cc Carbon disulfide during 1hours and mixed with magnetic stirrer. The reaction mixture was refluxed for 24 houre then kept for about 2 houre and cooled to the room temperature. It was filtered, washed with distilled water and dried

at room temperature name product was 3-methyl 5- mercupto 1,2,4-triazole thio carbohydrazid next stage ,To a thio carbohydrazid precipitate (10/6 gr) was added 50 cc acetic acid This mixture was refluxed for 2 hours , then cooled light solution and boiling.It was filtered, washed with distilled wate and dried at room temperature, yielded a white precipitate. TO a precipitate product was added para-hydroxy benzene aldehyde, in the molar ratio 1:1 before Chloroform solvent, mixed with magnetic stir bar then it was filtered, washed with ethanol and dried.



HBAMDT

Figure 1: Chemical structure of HBAMDT

.

Analysis of HBAMDT Ligand

Mp 275-278 °C, ¹HNMR (DMSO):6.8-7.7 (CH phenol), 10.3 (OH), 2.5(CH_3) 9.5(NH),8.2(CH azomethin) FT-IR (KBr,cm-1):1594s, 1115m. UV-vis (DMSO): λmax 260nm(ε26000), 290nm(ε26000) HBAMDT is soluble in acetone, acetonitrile DMF and DMSO and not soluble in water,ethanol and methanol

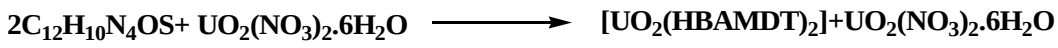
Analysis of NBAMDT ligand

Mp 232-2234 °C, ¹HNMR (DMSO):7-8.8 (CH nitro phenyl), 2.3(CH_3) 10.5(NH), 9.2(CH azomethin) FT-IR (KBr, cm^{-1}):1530s, 1094m. UV-vis (DMSO): λmax 265nm(ε 32000),310nm(ε 8000).

Synthesis of the [UO_2 (HBAMDT) $_2$] Complexes

A solution of uranyl (VI)nitrate salt dissolved in acetonitrile was added gradually to a Stirred with magnetic stirrer bar acetonitrile solution of the ligand(HBAMDT), in the

molar ratio 1:1 (metal:ligand). The reaction mixture was further stirred for 3 hours to ensure the completion and precipitation of the formed complexes. The precipitated solid complexes were filtered and washed several times with diethyl ether to remove any traces of the unreacted starting materials.



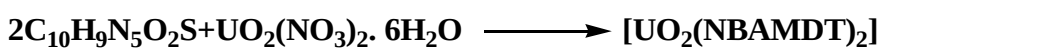
Equation 1

Analysis of $[UO_2(HBAMDT)_2]$

Yield, 80%. Anal. Calcd of $[UO_2(HBAMDT)_2]$,¹ HNMR (DMSO): 6.9-7.7 (CH phenol),10.2 (OH) , 2.5(CH3)),8 (CH azomethin) FT-IR (KBr,cm-1):1528s 741m,946s,476w,627m UV-vis (DMSO): λmax 355nm(ε 32000), 425nm(ε13000) [UO2(HBAMDT)2] is soluble in acetone,DMF and DMSO and not soluble in water,choloroform and methanol.

Synthesis of the $[UO_2(NBAMDT)_2]$ complex

NBAMDT ligand (1/6 gr) was solved in aceto nitrile (10 ml),obtained white color Solution,then uranylenitrate($UO_2(NO_3)_2.6H_2o$)(1.5gr)was solved in acetonitril(10ml).yellow color solution salt was added on the ligand solution and stirring with magnetic stirrer bar , was added salt on ligand and was immediate brown mixture. After 3 hours stirring precipitate washed with acetonitrile.



Equation 2

Analysis of $[UO_2(NBAMDT)_2]$ complex

Yield , 80%. Anal. Calcd of $[UO_2(NBAMDT)_2]$ ¹HNMR (DMSO): 7.9-8.7(CH nitrophenil)2.3(CH_3)),9.3(CHazomethin)FT-IR(KBr,cm-1) :1503s ,751m. 944s,5490w,626m UV-vis (DMSO): λmax

260nm(ε28000), 300nm(ε8000) ,380nm(ε3000) $[UO_2(NBAMDT)_2]$ is soluble in DMSO and dicholoro methan and not soluble in water Acetonitril,methanol,hexane and choloroform.

Cytotoxicity Studies

HBMADT ligand and $[UO_2(HBMDT)_2]$, $[UO_2(NBAMDT)_2]$ complexes Are two 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 (IC50), 190 mL of cell suspension 4×105 cell/mL) 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 periods 72hours for all cell lines, the cell concentrations were determined both in control and in drug-treated cultures. All experiments were done for six times.

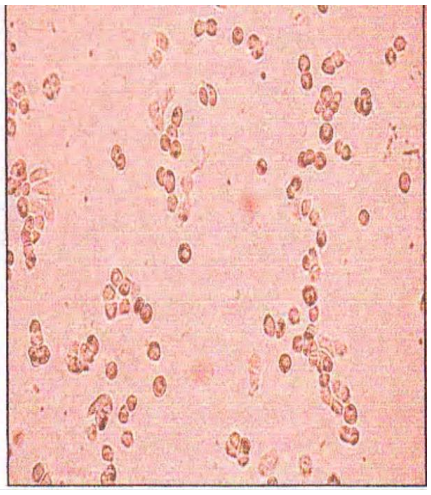


Figure 2: (a) morphology cells HT-29 after 72 .in cantain $[UO_2(HBMDT)_2]$(0.001M)

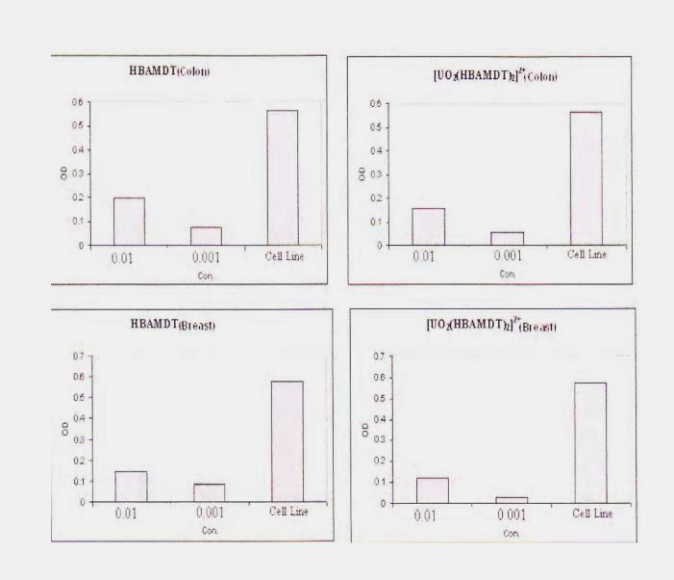


Figure3: concentrations several effect HBAMDT ligand and $[UO_2(HBMDT)_2]$complex

Results and Discussion

Preparation for Ligand, HBMDT, $[UO_2(HBMDT)_2]$ and $[UO_2(NBAMDT)_2]$ Complexe

The reaction of uranyl nitrate salts with the ligand in acetonitryl solvent results in the formation of $[ML_2]$ for M =U and L=HBMDT,NBMDT in the molar ratio 1:2(metal:ligand). All complexes are quite stable and could be stored without any appreciable change . All complexes were characterized by several techniques using FT-IR, electronic spectra NMR, UV-ViS and TG.The complexes $[UO_2(HBMDT)_2]$, $[UO_2(NBAMDT)_2]$ have 110-112 °C and223-225 °C melting point respectively . They are insoluble in common organic solvents, such as ethanol methanol, chloroform however, they are soluble in DMSO. Their structures were characterized by^{1}HNMR,^{13}CNMR,FT-IR and UV-ViS. The spectral data of the complexes have good relationship with the literature data.

Cytotoxicity Assays in Vitro

(HBMDT) and NBAMDT ligand and $[UO_2(HBMDT)_2]$and $[UO_2(NBAMDT)_2$ complexes have been tested against two human cancer cell lines: HT29 and T47D. The IC50. cytotoxicity values of the 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 [19]. The general method used for testing on antitumor properties of these compounds is thestandard testing method that has been previously described in greater detail .After preincubation lasting for 12(24) hours at 37C in 5٪ CO2 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 IC90 and IC50 of the dead cells and live cells were measured by trypan blue. The mechanism by which these complexes act as antitumor agents is apoptosis. IC90and IC_{50}values that are the compounds concentrations lethal for90% 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 doses (IC_{50} and IC_{90}) values are shown in Table 1.

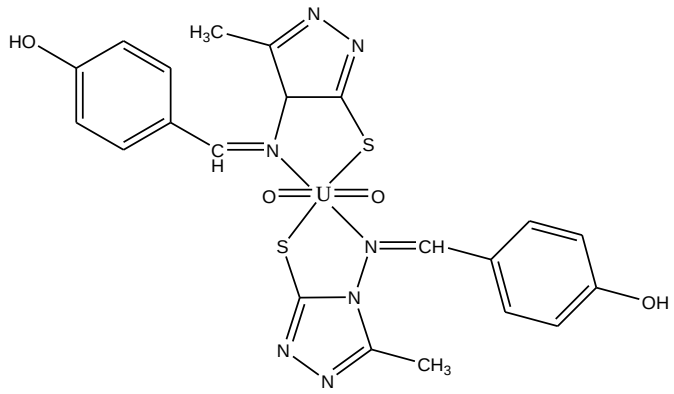
Based on the analytical data and physicochemical properties, the structure of complexes of salicylaldehyde-derivatives is proposed in which uranyl ion is coordinated through azomethine nitrogen and oxygen of the ionized phenolic hydroxyl group and the Schiff bases form a ter tridentate cleft (N_2O_2). Hydrazone derivatives and heterocyclic triazole derivatives are coordinated to the central metal atom as bidentate NN and NS ligands respectively. The results of simultaneous TG-DTG-DTA analyses of the complexes show the final degradation product for studied complexes are UO3 oxide.

Also the results show chelation causes drastic change in the biological properties of the ligands and also the metal moiety. So using chelating agent and complexation of the potentially multidentate ligands can prevent the toxic effects of uranyl.

Conclusion

It is clear from the above discussion that $[UO_2(HBMDT)_2]$and $[UO_2(NBAMDT)_2$ complexes and (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.structures two complexes $[UO_2(HBMDT)_2]$and $[UO_2(NBAMDT)_2$ suggest by ^{1}HNMR,^{13}CNMR,FT-IR and UV-ViS



$UO_2(HBMDT)_2$

Figuer4: Chemical structure of $[UO_2(HBMDT)_2]$

Acknowledgments

We gratefully acknowledge the financial support from the Research Council of Ardabil Islamic Azad University and many technical supports that are providedby Tarbiat Modarres University.

References

[1] X. F. Luo, X. Hu, X. Y. Zhao, S. H. Goh, and X. D. Li, Miscibility and interactions in blends and complexes of poly(4-methyl-5-vinylthiazole) with proton-donating polymers, *Polymer*., **44**, (18), 5285–5291(2003).

[2] A. S. N. Murthy and A. R. Reddy, Electronic absorption spectroscopic studies of enolimine-ketoamine equilibria in Schiff bases, *J. of Chem. Scie*., **90**(6), 519–526(1981).

[3] V. Razakantoanina, N. K. P. Phung, and G. Jaureguiberry, Antimalarial activity of new gossypol derivatives, *Parasitology. Res*., **86**(8), 665–668(2000).

[4] R. E. Royer, L. M. Deck, T. J. Vander Jagt, et al, Synthesis and anti-HIV activity of 1,1_-dideoxygossypol and related compounds, *J. of Med .Chem.*, **38**(13), 2427–2432(1995).

[5] M. R. Flack, R. G. Pyle, N. M. Mullen, et al, Oral gossypol in the treatment of

metastatic adrenal cancer, *J. of Clin. Endoc & Meta*., **76**, (4), 1019– 1024(1993).

[6] R. Baumgrass, M. Weiwad, F. Erdmann, et al, Reversible inhibition of calcineurin by the polyphenolic aldehyde gossypol, *J. of Biolog. Chem*., **276**(51) 47914–47921(2001).

[7] M. B. Teimouri and R. Bazhrang, Shaken not stirred: a facile synthesis of 1,4-bis(furo[2, 3, d]-pyrimidine-2,4(1H, 3H)- dione-5-yl)benzenes by one-pot reaction of isocyanides, N,N_-dimethylbarbituric acid, and terephthaldialdehyde, *Bioorg & Med. Chem .Lett*., **16**(14),3697–3701(2006).

[8] M. B. Teimouri, One-pot three-component reaction of isocyanides, dialkyl acetylenedicarboxylates and phthalhydrazide: synthesis of highly functionalized 1H-pyrazolo[1, 2− b]phthalazine-5,10-diones, *Tetrahedron.*, 62(47), 10849–10853(2006).

[9] J. V. Metzger, A. R. Katritzky, W. Rees, and K. T. Potts, *Eds, Compr. Hetero. Chem*., 6, Pergamon, Oxford, UK, 1984.

[10] J.M.Grevy, F. Tellez, S. Bern´es, H. N¨oth, R. Contreras, and N. Barba-Behrens, Coordination compounds of thiabendazole with main group and transition metal ions, *Inorg. Chimi. Acta*., **339**, 532–542(2002).

[11] A. Bernalte-Garc´ıa, F. J. Garc´ıa-Barros, F. J. Higes-Rolando, and F. Luna-Giles, Coordination chemistry of thiazoline/ thiazolidine derivatives. I: crystal structure of 2-(2-pyridyl)imino-N-(2-thiazolin-2-yl)thiazolidine (PyTT) and study of its interaction with nickel(II), Polyhedron., **18**(22), 2907–2912(1999).

[12] K. Lemma, J. Berglund, N. Farrell, and L. I. Elding, Kinetics and mechanism for reduction of anticancer-active tetrachloroam(m)ine platinum(IV) compounds by glutathione, *J. of Biolog. Inorg. Chem*., **5**(3), 300–306(2000).

[13] M. J. M. Campbell, Transition metal complexes of thiosemicarbazide and thiosemicarbazones, *Coordin. Chem. Rev*., **15**, (2-3), 279 319(1975).

[14] S. Padhy´e and G. B. Kauffman, Transition metal complexes of semicarbazones and thiosemicarbazones, *Coordin. Chem. Rev*., **63**,127–160, (1985).

[15] B. Erwin and C. Omoshile, Hydrogen isotope exchange in PtII-thiazole complexes, *J. of the Chem. Soc. Per. Trans*., **2**, 1333–1338(1995).

[16] G. Zhao, H. Lin, S. Zhu, H. Sun, and Y. Chen, Dinuclea palladium(II) complexes containing two monofunctional [Pd(en)(pyridine)Cl]+ units bridged by Se or S. Synthesis, characterization, cytotoxicity and kinetic studies of DNAbinding, *J. of*

Inorg. Biochem., **70**(3-4), 219–226(1998).