ISSN : 0974 - 746X Volume 6 Issue 1

Trade Science Inc.

Inorganic CHEMISTRY

An Indian Journal

Full Paper

ICAIJ, 6(1), 2011 [15-22]

Synthesis, characterization and anticancerous properties of mixed ligand Pd(II) and Ag(I) complexes with 2-amino-7-oxo-4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile and 2,2'-bipyridyl

A.M.Abdelghay[1]*, R.R.Zaky[2]

[1]Chemistry Department, Faculty of Science, Mansoura University, Mansoura, 35516, (EGYPT)

[2]Spectroscopy Department, Physics Division, National Research Centre, Cairo, (EGYPT)

E-mail : a.m_abdelghany@yahoo.com

Received: 3rd December, 2010 ; Accepted: 13th December, 2010

ABSTRACT

Two new water-soluble mixed ligand of$[Pd(bpy)(L)]Cl_2$ and$[Ag(bpy)(L)]NO_3$ complexes with 2-amino-7-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (L) were synthesised. The composition of the isolated complexes was discussed on the bases of elemental analysis, spectral studies (IR, UV–visible, ^{13}C-NMR and MS), as well as conductivity measurement. The synthesised complexes display a significant anticancer activity against *Ehrlich ascites* tumour cells (EACs). The higher activity of these complexes with their higher conductivity values corresponds to their complete ionization in aqueous solution.

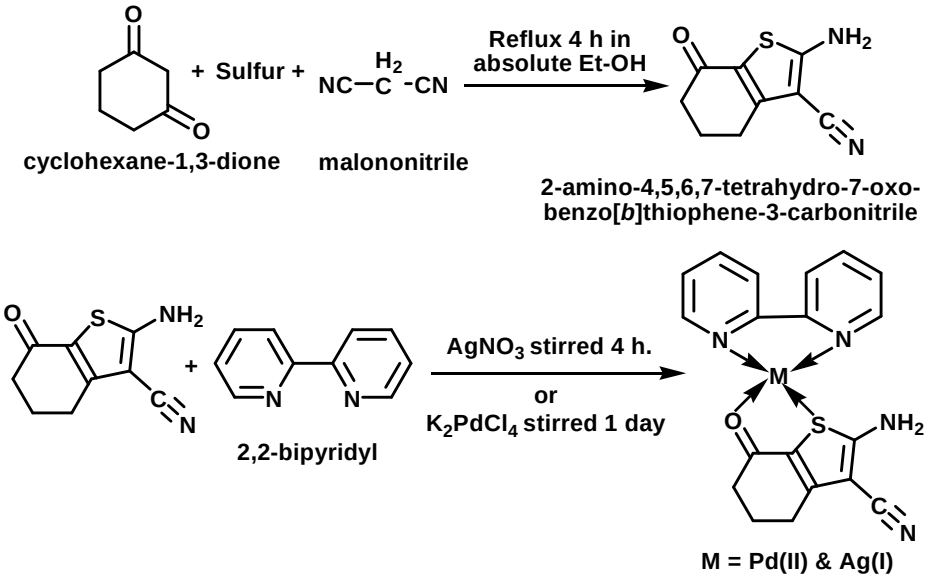
KEYWORDS

Mixed ligand complexes;
^{13}C-NMR;
Conductivity;
Anticancer activity.

INTRODUCTION

In the pharmaceutical industry, the discovery of new drugs requires an extensive safety and efficiency investigation before they are released into the market[1]. The safety and efficiency investigation is usually evaluated by toxicology data, which provides good insight into the drug's likely risk or benefit assessment in new drug application and also be interpreted in the assessment of health risk to people[2]. A variety of toxicological tests and clinical safety evaluations need to be conducted and evaluated by the drug regulatory authorities for drug safety assessment[3]. The use of computational methods is an efficient tool for identifying and illustrating unsafe drug candidates in the early stages of drug development, and it is particularly useful in predicting toxicological properties, such as genotoxicity, mutagenicity, hepatotoxicity, teratogenicity, and carcinogenicity. As vivo and vitro toxicity testing require a substantial investment in both time and money, these methods can reduce the cost and work force effectively. To develop a predictive toxicity model, the key step is to select a representative data set that associated with a predominating toxic mechanism. Thiophen is a sulphur-containing heterocyclic hydrocarbon that has been detected in a number of environmental sources as various derivatives[4-8]. Thiophen derivatives were selected due to their presence in pharmaceuticals and their potential, via cytochrome P450 (CYP450) metabolism. Thiophen is oxidized enzymatically by members of the CYP450 multifunction oxidises, resulting in the formation of thiophene epoxides and sulfoxides. These species are

Full Paper



Scheme 1 : The proposed structure of $[Pd(bpy)L]Cl_2$ and $[Ag(bpy)L]NO_3$

susceptible to nucleophilic attack by DNA nucleobases to form DNA adducts. When the damaged DNA is replicated, mutations occur in the newly formed DNA molecules as a result of the damaged parent DNA strand[9,10]. In a word, it will induce permanent gene mutations and may induce tumours in the end. So it is important to investigate the genotoxicity of thiophen derivatives using the machine learning methods. Machine learning methods have recently been explored using several approaches to predict toxicity of drugs[11-14].

The present work aims to synthesize and characterize complexes obtained from the reaction of 2-amino-7-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (L) with $[Pd(bpy)Cl_2]$ and $[Ag(bpy)(H_2O)_2]NO_3$. They have been investigated using elemental analysis, spectral studies (IR, UV-visible, ^{13}C-NMR and MS), as well as conductivity measurement. In addition, the anticancer activity of these complexes.

EXPERIMENTAL

Material and methods

All manipulations were performed under aerobic conditions. All metal salts and other reagents used were pure (Merck). $[Pd(bpy)Cl_2]$ was synthesized by the literature method. The cells of *Ehrlich ascites* (EACs) tumour were obtained from National Cancer Institute (Cairo, Egypt). After harvesting and preparation of the cells, their total number and viability were determined by counting using Trypan blue[15].

Instrumentation

Electronic spectra were recorded using a Unicam UV_{2-100} UV-Vis. Spectrometer. IR spectra were measured as KBr discs on a Matson 5000 FT-IR spectrometer. ^{13}C-NMR spectra were measured on a Varian Gemini WM-200 spectrometer. Conduct metric measurements were carried out at room temperature on an YSI Model 32 conductivity bridge. Mass spectra were recorded on a Matson MS 5988 spectrometer.

Synthesis of 2-amino-7-oxo-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile

2-amino-7-oxo-4, 5, 6, 7-tetrahydrobenzo [*b*]thio- phene-3-carbonitrile (L) was prepared by heating a mixture of cyclohexane-1,3-dione (0.01 mol; 1.12 g) with *malononitrile* (0.01 mol; 0.66 g) and sulphur (0.01mol; 0.32 g) under reflux in absolute ethanol for 4 h. On heating, yellow crystals were formed, filtered off, washed with hot ethanol and diethyl ether, then recrystallized from ethanol (M.p.: 175°C; yield 90%). The purity of the compound was checked by TLC (Scheme 1).

Synthesis of complexes

(1) Synthesis of $[Pd(bpy)L]Cl_2 \cdot H_2O$

A stirred suspension of $[Pd(bpy)Cl_2]$ (0.17g, 0.5 mmol) in methanol-benzene (3 : 2, V/V) (15 cm^3), was

added a methanolic solution of KOH (0.055g, 1mmol) containing 2-amino-7-oxo-4, 5, 6, 7-tetrahydrobenzo [*b*] thiophene-3-carbonitrile (L) (0.096g, 0.5mmol). The resulting suspension was stirred for one days and a brown complex was obtained. It was filtered off, washed with water and methanol, and then air-dried. Conductivity data (10^{-3}M in DMF): Λ_M = 89.0 ohm^{-1}cm^{2}mol^{-1}. Elemental Anal. Calc. for $C_{19}Cl_2H_{16}N_4OSPd$: C, 43.41; H, 3.07; N, 10.66; Cl, 13.49; Pd, 20.24. Found C, 43.53; H, 3.11; N, 10.51; Cl, 13.36; Pd, 19.92. (525.7544).

(2) Synthesis of [Ag(bpy)L]NO_3

Silver nitrate (0.087g, 0.5mmol) in water (2cm^3) was added to bpy (0.078g, 0.5mmol) in methanol (35cm^3) to produce a colourless solution, to which L (0.096g, 0.5mol) was added. The reaction mixture was stirred in dark for 4 hours to produce a faint brown solid. It was filtered off, washed with little water, methanol, and diethyl ether, then dried in vacuo. Conductivity data (10^{-3} M in DMF): Λ_M = 68.0 ohm^{-1}cm^{2}mol^{-1}. Elemental Anal. Calc. for $C_{19}H_{16}N_5O_4SAg$: C, 44.03; H, 3.11; N, 13.51; Ag, 20.81. Found C, 44.22; H, 3.18; N, 13.42; Ag, 21.01. (518.3014).

Conductivity measurements

The conductivity values for[Pd(bpy)L]Cl_2 and[Ag(bpy)L]NO_3 were determined. The compounds were dissolved in water and the measurements were done at concentrations; 1, 0.8, 0.6, 0.4, and 0.2 mM. The conductance values (Λ_M) were calculated and plotted against concentration[16].

Anticancer activity against ehrlich ascites carcinoma in mice

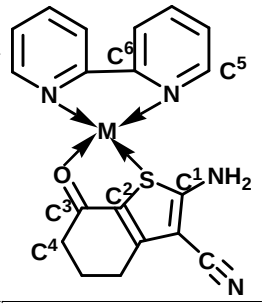
All the compounds were screened for their anticancer activity by dissolving samples in minimum amount of DMSO (L) or water (complexes) and diluting with phosphate-buffered saline (PBS; pH = 7.2). The anticancer studies using *Ehrlich ascites* tumour cells (EACs) were carried out by incubating 0.2mL of cells IP. All the treatments started 24 hours after inoculation for 45 days. The tumor-bearing mice were divided into three groups. Group (1) is the standard one that received the 5-florouracil[17] (5-fu; 20mg/kg/day of mice) for comparison. Group (2) received L, [Pd(bpy)L]Cl_2, and [Ag(bpy)L]NO_3 complexes (0.01mg/mice/day).

TABLE 1 : Spectral data of L and its complexes

Compound	ν(NH$_2$)	ν(C=O)	ν(CS)	ν(CN)	ν(M–O)	ν(M–S)
L	3360	1675	1155	2226	-	-
[Pd(bpy)(L)]Cl_2	3369	1643	1143	2228	553	390
[Ag(bpy)(L)]NO_3	3374	1649	1140	2231	550	381

TABLE 2 : ^{13}C-NMR Chemical shift in (ppm) assignments for L, [Pd(bpy)L]Cl_2 and [Ag(bpy)L]NO_3 in DMSO

Assignment	L	[Pd(bpy)L] Cl_2	[Ag(bpy)L] NO_3
C^1	148	142	144
C^2	165	161	160
C^3	182	178	176
C^4	60	55	55
C^5	142	149	147
C_6	146	152	151



Group (3) is the control one received physiological saline (0.9% sodium chloride).

RESULTS AND DISCUSSION

Synthesis of complexes

The two new complexes of 2-amino-7-oxo-4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (L) see Scheme 1. The elemental analyses of the isolated complexes agree with the assigned formula. The conductivities (Λ_M) in DMF at room temperature showed the electrolytic character of these complexes[17]. The complex [Pd(bpy)L]Cl_2 was prepared from [Pd(bpy)Cl_2] and (L) in methanol-benzene in presence of aqueous base, while [Ag(bpy)L]NO_3 was prepared from aqueous $AgNO_3$ with bpy and L in methanol.

The complexes are powder-like, stable in the normal laboratory atmosphere, and soluble in water, DMF or DMSO. We had hoped to characterize the structure of one of the complexes by single X-ray crystallography, but were thwarted on numerous occasions by very small crystal dimensions. Thus, the characterization of these complexes was based on the physical and spectroscopic techniques.

Vibration spectra

The characteristic IR bands observed and vibration assignments of 2-amino-7-oxo-4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (L) complexes are reported in TABLE 1. The infrared spec-

TABLE 3 : Haematological and biochemical parameters of (L) and its complexes

Compound	Parameters					
	Hb[(1)] 12-16 g/dl	RBCs[(2)] 4.06×10^6 cell/cm^3	HCT[(3)] 35.0-50%	WBCs[(4)] $4000\text{-}11000 \times 10^6$ cell/cm^3	EAC Count ($\times 10^6$ cell/cm^3)	MsT/Day[(5)]
L	10.9	6.4	41.3	8700	41.2	13.0
[Pd(bpy)L]Cl2	10.9	6.2	39.3	12000	32.5	10.8
[Ag(bpy)L]NO3	10.8	6.4	42.0	9800	35.1	10.9
5-fu	10.2	6	37.7	7600	80	13.6
Control	7.8	4.72	22.2	2400	220	9.0

[(1)]Hb = haemoglobin, [(2)]RBCs = red blood cells count, [(3)]HCT = hemato crate value, [(4)]WBCs = white blood cells count values in normal mice are in parentheses, [(5)]the mean survival time

trum of (L) displays four bands at 3360, 1675, 1155, and 2226 cm^{-1} assigned to $\upsilon(NH_2)$[18], $\upsilon(C=O)$[19], $\nu(CS)$[20] and $\nu(CN)$[21] vibrations, respectively. By comparison the infrared spectrum of the ligand with those of its metal complexes reveals that (L) behaves as a neutral bidentate ligand coordinating via carbonyl oxygen (C=O) and thiophen sulphur (CS). This mode of complexation is supported by the shift of both ν(C=O) and ν(CS) vibrations to lower wavenumber. Also, the bands at 3360 and 2226 cm^{-1}, arising from $\nu(NH_2)$ and ν(CN), respectively, are not affected upon complexation. The infrared spectra of these complexes show new bands at 553, 550 and 390, 381 cm^{-1} assignable to ν(M–O) and ν(M–S), respectively[22]. Moreover, the bands of the free bpy ligand near 749cm^{-1} are shifted to higher frequencies in the complexes (776 cm^{-1})[23]. The spectrum of[Ag(bpy)(L)]NO_3 shows new strong band near 1373cm^{-1} assigned to the ionic uncoordinated NO_3^-[24].

Electronic spectra

The electronic spectrum of[Pd(bpy)L]Cl_2 complex (Figure 1) shows bands at 492 and 346 nm due to $^1A_{1g} \rightarrow {}^1B_{1g}$ and $^1A_{1g} \rightarrow {}^1E_{1g}$, transitions in a square-planar configuration[25]. Also, the spectrum of [Ag(bpy)L]NO_3 complex (Figure 1) shows bands at 485, 380, and 286 nm; the latter two may arise from charge transfer of the type ligand (π) $\rightarrow b_{1g}$ (Ag^+) and ligand (σ) $\rightarrow b_{1g}$ (Ag^+), respectively, in a typically distorted square planar environment around the metal ion[26].

Nuclear magnetic resonance spectral studies

The ^{13}C-NMR spectra of (L) and its Pd(II) and Ag(I) complexes (Figure 2) were recorded in DMSO. The most significant features of the ^{13}C-NMR spectra of the complexes were detected when comparing with the spectrum of the corresponding free ligand. As expected, C^1, C^2, C^3 and C^4 carbons showed an upfield shift, but, C^5 and C^6 carbons showed downfield shift on complexation[27,28]. The other ring carbon atoms did not show significant shifts (TABLE 2).

Mass spectra

The mass spectra for the two complexes and the molecular ion peaks confirmed the proposed formulae. As a typical example, the mass spectrum (Figure 3) of [Pd(bpy)L]Cl_2 shows peaks corresponding to the successive degradation of the molecule. The first peak at m/e 526.00 (Calculate. 525.75) represents the molecular ion peak of the complex (M^{+2}). The sharp peak (base peak) with m/e 107 represents the stable and final residue Pd(II).

Conductivity measurements

Figure 4 shows the plots of the conductivities of [Pd(bpy)L]Cl_2 and[Ag(bpy)L]NO_3 against concentrations. It is clear that as the concentration increases, the conductivity increases, indicating the complete ionization of the complexes species[29]. Since the conductivity for Cl^- and NO_3^- is 76 and 71 ohm cm^2, respectively[30], this suggests that the complexes ionized completely in aqueous media[31].

Anticancerous activity

The reliable criteria for judging the efficacy of any anticancer drug are prolongation of life span, improving the clinical, haematological, and biochemical profile, as well as reduction in viable tumour cell count in the host[32-34]. It is known that the anticancer available drugs inhibit the haematological and biochemical parameters (haemoglobin (Hb), red blood cells count (RBCs), and white blood cells count (WBCs); blood picture). The

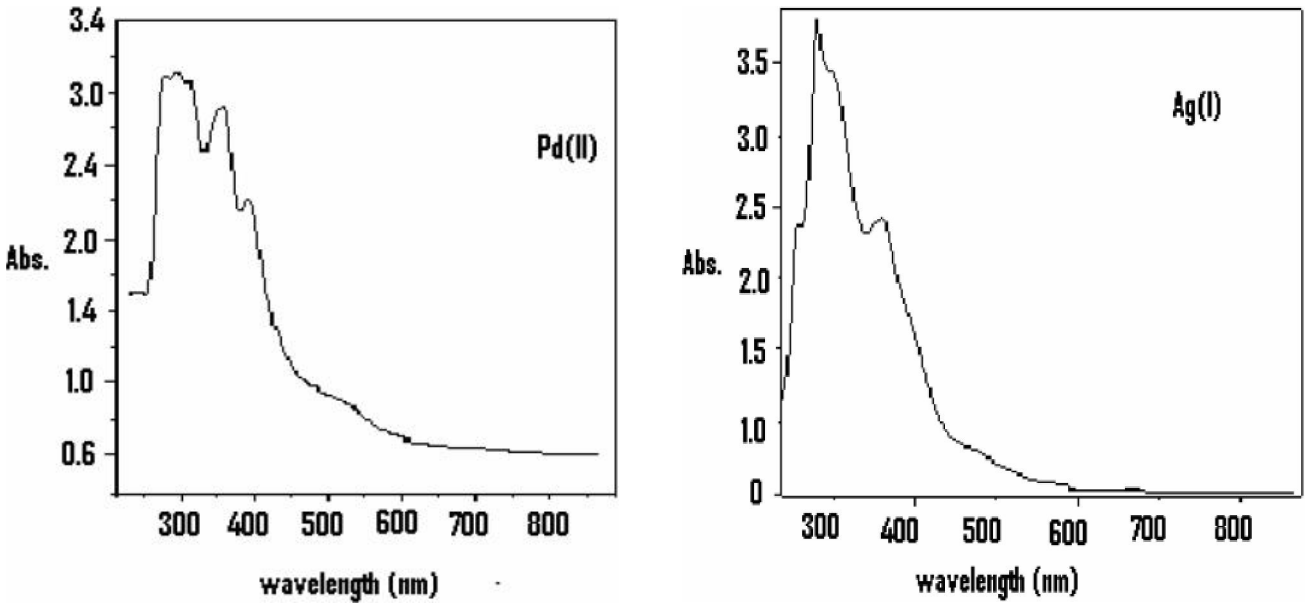


Figure 1 : Electronic spectra of $[Pd(bpy)L]Cl_2$ and $[Ag(bpy)L]NO_3$

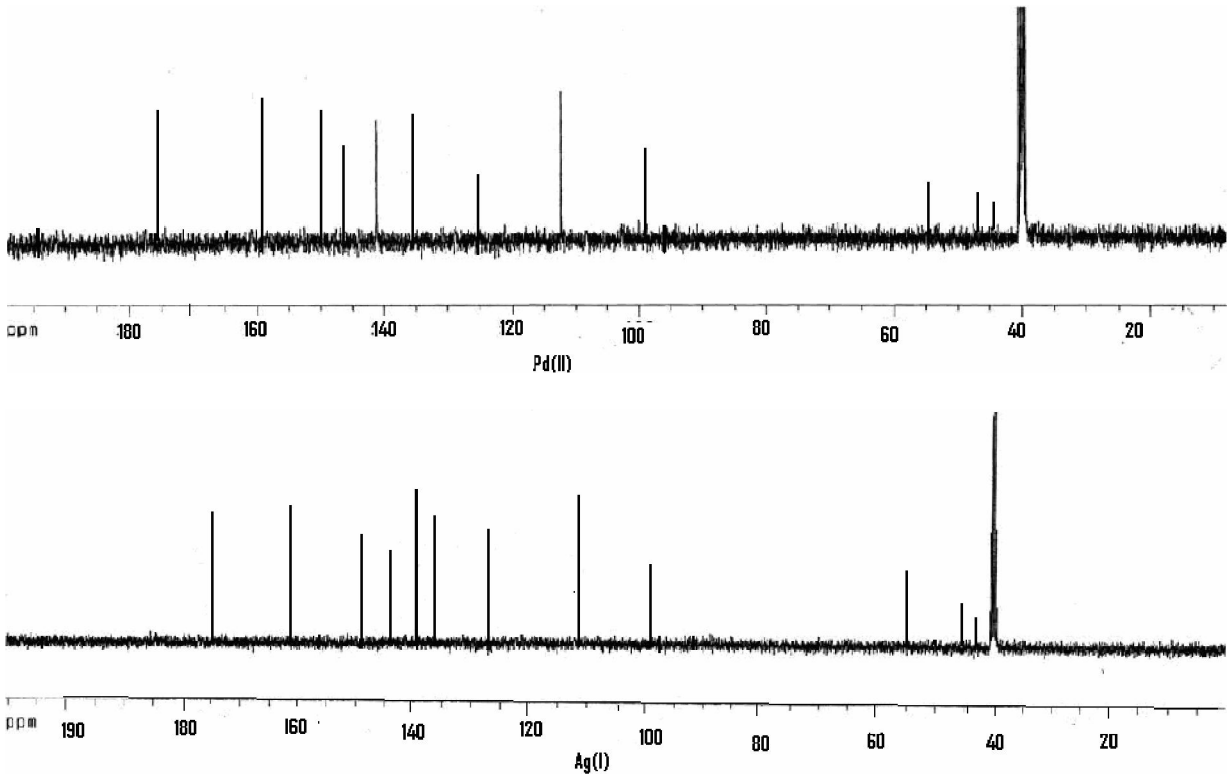


Figure 2 : ^{13}C-NMR Chemical shifts of $[Pd(bpy)L]Cl_2$ and $[Ag(bpy)L]NO_3$

ultimate goal of this project is to develop mixed ligand complexes containing nitrogen bases effective against cancer without side effects on the haematological and biochemical parameters.

In order to detect the influence of (L), $[Pd(bpy)L]Cl_2$ and $[Ag(bpy)L]NO_3$ on the haemato- logical status of EAC-bearing mice, a comparison study was made among three groups of mice (each group contains seven mice) from the second day after inoculation. Group (1) tumor-bearing mice treated with 5-fu (standard[35,36]). Group (2) tumor-bearing mice treated with (L), $[Pd(bpy)L]Cl_2$, and $[Ag(bpy)L]NO_3$. Group (3) is the

Full Paper

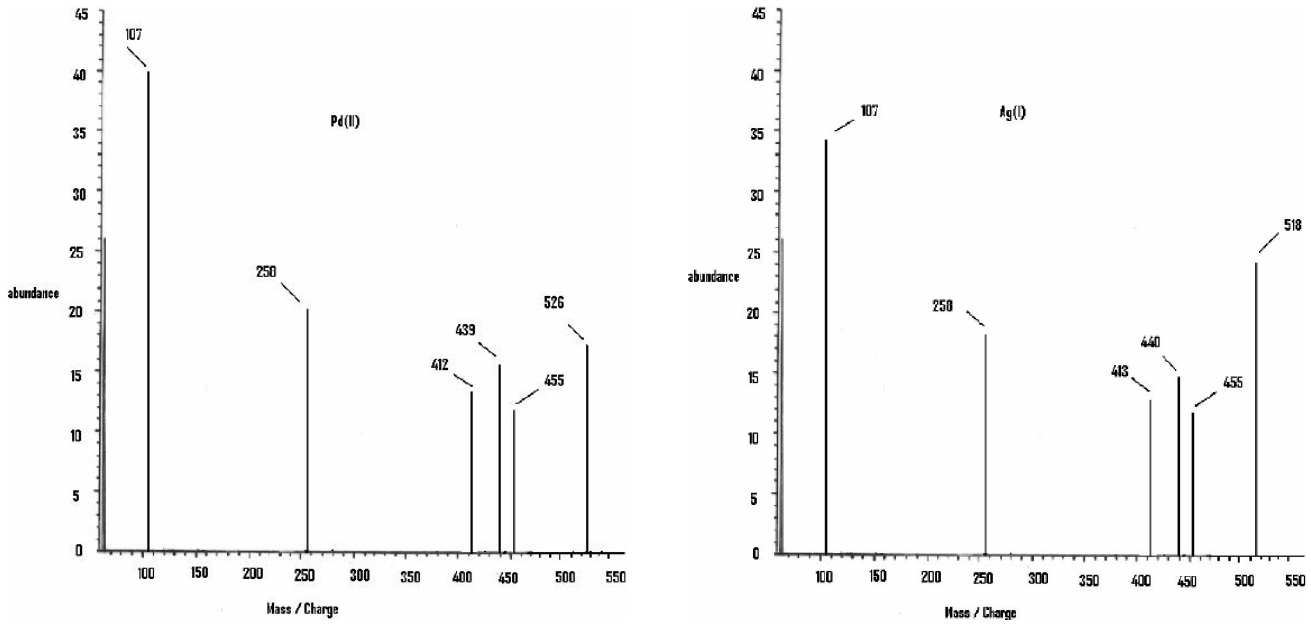


Figure 3 : Mass spectra of $[Pd(bpy)L]Cl_2$ and $[Ag(bpy)L]NO_3$

control tumor-bearing mice. The anticancer activity of L, $[Pd(bpy)L]Cl_2$, and $[Ag(bpy)L]NO_3$ shows remarkable efficacy manifested by survival and activity, as well as reduction in the tumour size. The haematological parameters including haemoglobin (Hb), red blood cells count (RBCs), and white blood cells count (WBCs) data are reported in TABLE 3. It is clear that the haematological parameters of tumor-bearing mice treated with (L), $[Pd(bpy)L]Cl_2$, and $[Ag(bpy)L]NO_3$ exhibits much better significant figures with the use of small doses of (0.01 mg/mice/day) compared with the standard (5-fu), the market drug (~0.4 mg/mice/day).

There are reports that complexes containing pyridine ring (cyclic nitrogen) display significant anticancer activity[37]. Thus, the presence of the pyrimidine ring increases the anticancer activity and activates the binding of metal ion to the tumour DNA as it contains two cyclic nitrogen atoms[34].

The haematological parameters show that $[Pd(bpy)L]Cl_2$ and $[Ag(bpy)L]NO_3$ are more effective than (L) itself, as the presence of both bpy and (L) in the complexes possess a multiring planar area with nitrogen bases and hence higher hydrophobicity, which would lead the intercalation more deeply into the tumour DNA[38].

In order to investigate the action of Pd(II) and Ag(I) complexes in the tumour DNA, the intercalated complexes affecting the structure of the DNA prevent polymerase and other DNA binding proteins from functioning properly. As the complexes covantely bind to DNA with preferential binding to the N-7 position of guanine and adenine, they are able to bind two different sites on DNA, producing cross-links that cause increase in the viscosity in comparison to the normal unbound DNA. The results are prevention of DNA synthesis, inhibition of transcription, and induction of mutations[38].

Regarding the tumour size and EAC count, in the control group was (220×10^6 cells/cm^3), reduced in using 5-fu to (80×10^6 cells/cm^3) while the strong reduction to 41.2×10^6, 32.5×10^6, and 35.1×10^6 cells per cm^3 was observed in using (L), $[Pd(bpy)L]Cl_2$, and $[Ag(bpy)L]NO_3$, respectively. The strong reduction in EAC count and tumour size may be due to the reductive nature of many tumours that contain significant regions at low oxygen tension. Thus, they initiate a catalytic auto-oxidation process involving generation of reactive oxygen species. The coordinated (L) and bpy may reduce toxic effects caused by xenobiotic core of the complexes, contribute to their anticancer action, and facilitate their transport through cell membrane[39]. Our investigations have shown that glutathione content, in liver and kidney, and glutathione-S-transferase activity were decreased, suggesting that the partial reduction products of oxygen, in the presence of the complexes, yield very reactive species, which could start catalytic oxidation of substrates and show antitumor action[40].

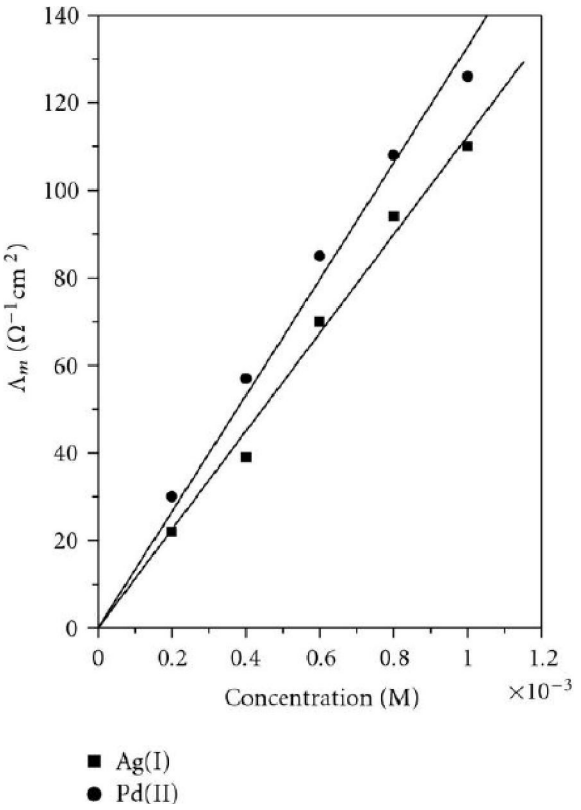


Figure 4 : Conductance-concentration relationship of the complexes

In order to detect the influence of the solvent in the cytotoxicity of (L), $[Pd(bpy)L]Cl_2$, and $[Ag(bpy)L]NO_3$,As expected, the water-soluble $[Pd(bpy)L]Cl_2$ and $[Ag(bpy)L]NO_3$ are less kidney toxic.

Effect of survival time

The mean survival time (MST) of groups 1 and 2 was compared with that of the control group using the following calculations[41]. Percentage (%) increase in lifespan over control =[(MST of treated group × 100/ MST of control group) -100]; MST = days of each mice in the group/total number of mice. Percentage (%) increase in lifespan over control showed to be high in mice treated with (L), $[Pd(bpy)L]Cl_2$, and $[Ag(bpy)L]NO_3$ (TABLE 3).

The side effects and toxicity

The side effects and toxicity of (L), $[Pd(bpy)L]Cl_2$ and $[Ag(bpy)L]NO_3$ have been detected. After the first week of the treatment, the mice show flu-like attack and in the third week show spot dropping on the hair (alopecia). Fortunately, the solid organs have not been affected.

CONCLUSION

There are reports that complexes containing pyridine ring (cyclic nitrogen) display significant anticancer activity. The anticancer activity of the new water-soluble complexes, $[Pd(bpy)L]Cl_2$ and $[Ag(bpy)L]NO_3$, shows remarkable efficacy against *Ehrlich ascites* tumour cells (EACs) manifested by survival and activity, as well as reduction in the tumour size.

REFERENCES

[1] D.Purves, C.Harvey, D.Tweats, C.E.Lumley; Mutagenesis Genotoxicity Testing, **10(4)**, 297-312 **(1995)**.
[2] L.He, P.C.Jurs, L.L.Custer, S.K.Durham, G.M.Pearl; Chem.Res.Toxicol., **16**, 1567-80 **(2003)**.
[3] T.Kennedy; Drug Discov.Today, **2**, 436-44 **(1997)**.
[4] A.D.Bolzan, M.S.Bianchi; Muta.Res.Rev.Mutat., **512(2-3)**, 121-34 **(2002)**.
[5] L.Arbillaga, A.Azqueta, J.H.M.V.Delft, A.L.D.Cerain; Toxicol.Appl.Pharmacol., **220(12)**, 216-24 **(2007)**.
[6] K.G.Kropp, P.M.Fedorak; Can.J.Microbiol., **44**, 605-22 **(1998)**.
[7] F.Fonnum, E.A.Lock; Toxicol.Lett., **212**, 9-16 **(2000)**.
[8] F.Habersetzer, D.Larrey, G.Babany, C.Degott, M.Corbic, D.Pessayre, J.P.Benhamou; J.Hepatol., **9**, 256-59 **(1989)**.
[9] T.Mizutani, K.Yoshida, S.Kawazoe; Drug Metab.Dispos., **22**, 750-55 **(1994)**.
[10] J.M.Machinist, M.D.Mayer, M.S.Shet, J.L.Ferrero, A.D.Rodriguez; Drug Metab.Dispos., **23**, 1163-74 **(1995)**.
[11] A.P.Freidig, S.Dekkers, M.Verwei, E.Zvinavashe, J.G.M.Bessems, J.J.M.Van de Sandt; Toxicol.Lett., **170**, 214-22 **(2007)**.
[12] F.P.Guengerich, J.S.MacDonald; Chem.Res. Toxicol., **20**, 344-69 **(2007)**.
[13] D.C.Liebler, F.P.Guengerich; Nat.Rev.Drug Discov., **4**, 410-20 **(2005)**.
[14] P.D.Mosier, P.C.Jurs, L.L.Custer, S.K.Durham, G.M.Pearl; Chem.Res.Toxicol., **16**, 721-32 **(2003)**.
[15] L.G.Menon, G.Kuttan, R.Kuttan; J.Exp.Clinic.Cancer Res., **15(3)**, 241-43 **(1996)**.
[16] W.J.Geary; Coord.Chem.Rev., **7**, 81-122 **(1981)**.
[17] A.Abdullah, F.Huq, A.Chowdhury, H.Tayyem, P.Beale, K.Fisher; BMC Chem.Biology., **3**, 1472-84 **(2006)**.

Full Paper

[18] S.I.Mostafa, S.A.Abd El-Maksoud; Monatshefte Fur Chemie., **129**, 455-66 **(1998)**.
[19] N.M.El-Metwally, I.M.Gabr, A.A.El-Asmy; Transit.Met.Chem., **31**, 71-78 **(2006)**.
[20] G.A.A.Al-Hazmi, M.S.EL-Shahawi, I.M.Gabr, A.A.EL-Asmy; J.Coord.Chem., **58**, 713-33 **(2005)**.
[21] K.H.Reddy, P.S.Reddy, P.R.Babu; J.Inorg. Biochem., **77**, 169-76 **(1999)**.
[22] S.Tollari, G.Palmisano, F.Demartin, M.Grassi, S.Magnaghi, S.Cenini; J.Organomet.Chem., **488**, 79-83 **(1995)**.
[23] R.Castro, J.A.G.Vazquez, J.Romero, A.Sousa, R.Pritchard, C.A.McAuliffe; J.Chem.Soc.Dalton Trans, **7**, 1115-20 **(1994)**.
[24] M.Abul Haj, M.Quiros, J.M.Salas, R.Faure; J.Chem.Soc.Dalton Trans, **11**, 1798-801 **(2001)**.
[25] S.I.Mostafa, M.M.Bekheit; Chem.Pharma.Bulle., **48**, 266-71 **(2000)**.
[26] F.Sabin, C.K.Ryu, P.Fork, A.Vogler; Inorg.Chem., **31**, 1941–46 **(1992)**.
[27] M.C.Rodríguez-Argüelles, P.Tourón-Touceda, R.Cao, A.M.García-Deibe, P.Pelagatti, C.Pelizzi, F.Zani; J.Inorg.Biochem., **103**, 35-42 **(2009)**.
[28] N.Raman, R.Jeyamurugan; J.Coord.Chem., **62(14)**, 2375-87 **(2009)**.
[29] W.P.Griffith, S.I.Mostafa; **11(23)**, 2997-3005 **(1992)**.
[30] D.Aguado, T.Montoya, J.Ferrer, A.Seco; **21**, 845-51 **(2006)**.
[31] G.W.Castellan; Phys.Chem., Menlo Park, Calif, USA, The Benjamin, **(1983)**.
[32] B.D.Clarkson, J.H.Burchenal; Prog.Clinic.Cancer., **2**, 353-423 **(1954)**.
[33] S.I.Mostafa; Transit.Met.Chem., **32**, 769-75 **(2007)**.
[34] B.Ardalan, M.D.Buscaglia, P.S.Schein; Biochem. Pharma., **27**, 2009-13 **(1978)**.
[35] K.Yoshisue, Z.Hironaga, S.Yamaguchi, A.Yamamoto, S.Nagayama, Y.Kawaguchi; Cancer.Chemothera.Pharma., **46**, 51-56 **(2000)**.
[36] A.Romerosa, P.Bergamini, V.Bertolasi; Inorg. Chem., **43**, 905-13 **(2004)**.
[37] S.I.Mostafa; J.Coord.Chem., **61(10)**, 1553-67 **(2009)**.
[38] S.Osinsky, I.Levitin, L.Bubnovskaya, I.Ganusevich, V.Tsikalova, Y.Istomin, E.Zhavrid, M.Volpin; Anticancer Research, **17(5A)**, 3457-62 **(1997)**.
[39] S.Osinsky, I.Levitin, L.Bubnovskaya, A.Sigan, I.Ganusevich, V.Mikhailenko, T.Kovelskaya; International Journal of Medicine, Biology and the Environment, **28(1)**, 83-87 **(2000)**.
[40] P.Sur, D.K.Ganguly; Planta.Medica., **60**, 106-9 **(1994)**.