

Synthesis and characterization of some metal complexes derived from *L*-lysine dihydrochloride with some metal ions by tribochemical reactions

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ABSTRACT

Four new complexes with the general formula, $R_2[MCl_4]$ ($R=L$ -lysine dihydrochloride), were synthesized by grinding *L*-lysine dihydrochloride and MX_2 in the solid state in a mortar by tribochemical reaction. The isolated complexes with the general formula, $R_2[MCl_4]$, derived from Cu^{2+} , Ba^{2+} , Cd^{2+} and Pd^{2+} chlorides are characterized by elemental analyses, conductivities, spectral (IR, UV-Vis, Far-IR) and magnetic measurements. Spectral and magnetic measurements suggest that the metal ions form tetrahedral geometry around the metal ion. The $[MCl_4]$ anion and its counter ions are connected through a hydrogen bonds between Cl of the anion and OH (carboxylate) forming $O-H \cdots Cl$ interaction. Molar conductance values of complexes suggest the electrolytic nature of these complexes in DMSO but easily dissociated in H_2O indicating that the hydrogen bond formed is very weak. The results of Far-IR spectra suggest the stretching and bending vibrations of M-Cl. Also, the isolated complexes were tested against different types of cancer and some the complexes give promising results. © 2015 Trade Science Inc. - INDIA

KEYWORDS

Tribochemical reactions;
Tetrahalometallate complexes;
Spectroscopic studies;
Far-FTIR;
Biological activity.

INTRODUCTION

The study of the biological role of metal ions has a long history in medicine, pharmacology, toxicology and recently the extent and variety of metal ions involvement have been appreciated. The metal behavior in *vivo* which cannot be over emphasized is essentially that of the complex ion. Properties such as the effective size and solubility of a metal ion in *vivo* are a function of ligand and solvent present as

well as of the metal ions themselves. The chemistry of these ions in *vivo* is that of ions which present in an excess of competing groups.

Tetrachloropalladate(II) complex with glutamine of the formula, $[PdCl_4][(L\text{-Glutamine})_2]$, was prepared and characterized via IR spectroscopy^[1]. The compound was found cytotoxic to TA98 and TA100 bacterial cells of salmonella typhimurium. The structure of {3-[(4-fluorophenyl)methyl]-1H-benzimidazol-2-ylidene} {1-[2-(4-methoxyphenyl) ethyl]-4-

piperidin-1-yl} ammoniumtetrachlorocuprate(II), $[\text{C}_{28}\text{H}_{33}\text{FN}_4\text{O}] [\text{CuCl}_4]$, was also investigated by Parvez et al^[2]. The geometry around Cu is flattened tetrahedral with significantly different Cu-Cl distances which lie in the range 2.1968(14)-2.2861(12) Å. The compound, $[\text{C}_8\text{H}_{22}\text{N}_2][\text{CuCl}_4]$, which was composed of one *N,N,N',N'*-tetramethylbutane-1,4-diammonium cation and a tetrachlorocuprate(II) anion was investigated by Elangovan et al^[3]. The anion was mononuclear and has a flattened tetrahedral geometry. Two new compounds, bis-(*DL*-erythro-2-piperidylum-2,8-bis(trifluoromethyl)-4-quinolinemethanol) tetrachlorocuprate (II) tetrahydrate, $[\text{LH}]_2[\text{CuCl}_4] \cdot 4\text{H}_2\text{O}$ ($\text{L} = \text{mefloquine}$) and bis(*DL*-erythro-2-piperidylum-2,8-bis(trifluoromethyl)quinolinemethanol) tetrabromocadmiate (II) bis(methanol) $[\text{LH}^+]_2 [\text{CdBr}_4]^{2-} \cdot 2\text{CH}_3\text{OH}$, were studied by Joshua et al^[4]. The two compounds were characterized by elemental analysis, ¹H-NMR and IR spectroscopy. Adams et al^[5] reported the reactions of Pd^{2+} and Pd^{2+} chloride complexes with imidazole and pyrazole or their hydrochloride in solid state. The salts are shown to produce metal complex salts and coordination compounds. Thus, $\text{K}_2[\text{MCl}_4]$ or MCl_2 can be ground with imidazolium chloride ($[\text{H}_2\text{im}]\text{Cl}$) to produce salts of the type $[\text{H}_2\text{im}]_2[\text{MCl}_4]$. The synthesis and crystal structure of thermochromic, yellow benzimidazoliumtetrachlorocuprate (II), $[\text{C}_7\text{H}_7\text{N}_2]_2 [\text{CuCl}_4]$, have been reported^[6]. The compound crystallizes in the $\text{C}2/c$ space group and contains discrete tetrahedral $[\text{CuCl}_4]^{2-}$ species. The role of the water molecule on the solid state, yellow \rightleftharpoons /green thermochromic transformation, was discussed. A series of new complex salts of the type $[\text{A}]_2[\text{MCl}_2\text{I}_2]$ where $\text{A} = 1,3,5$ -trimethylpyridinium cation, $\text{M} = \text{Mn}^{2+}, \text{Co}^{2+}, \text{Ni}^{2+}, \text{Cu}^{2+}$ and Zn^{2+} , were prepared by the reaction of the metal chloride and 3,5-trimethylpyridinium iodide in (1:2) molar ratio and characterized by elemental analysis, molar conductance, IR, UV/Vis., spectral studies and magnetic measurements^[7]. The crystal structure of 1,3,5-trimethylpyridinium iodide was determined by single crystal x-ray crystallography. The complex salts of the type $[\text{R}]_2[\text{MCl}_4]$ {where $\text{R} = [\text{CN}_4(\text{C}_6\text{H}_5)_3]_2$, $\text{M} = \text{Mn}^{2+}, \text{Co}^{2+}, \text{Ni}^{2+}, \text{Cu}^{2+}$ and Zn^{2+} }, were prepared and

characterized by elemental analysis, molar conductance, IR and UV/Vis spectral studies and magnetic measurements. The crystal structure of $[\text{CN}_4(\text{C}_6\text{H}_5)_3]_2[\text{CuCl}_4]_2$ was determined by single crystal X-ray crystallography^[8]. The structure consisted of anion part and 2,3,5-(triphenyl) tetrazolium cation as counter ion. The copper complex has a distorted-tetrahedral geometry and the $[\text{CuCl}_4]$ anion and its counter ions are connected through a hydrogen bonds between Cl of the dianion and hetero aromatic rings by Cl- π , π - π and O-H \cdots π , interactions.

In continuation of our earlier work^[9-13] we extend our study to synthesize new metal complexes derived from *L*-lysinedihydrochloride with some metal ions by tribochemical reactions. Also, one of our main goals of this work is to study the biological activity of the isolated complexes against different types of cancer.

EXPERIMENTAL

Materials and methods

Carbon, hydrogen and nitrogen contents were determined at the Microanalytical Unit, Cairo University, Egypt. The metal contents (Pd and Cd) were determined by complexometric titration using Xylenol orange as indicator^[14]. Cu and Ba contents were performed with AAS (flame absorption) model Perkin-Elmer in the Micro Analytical Center, Faculty of Science at Cairo University. The conductance measurements in DMSO were carried out using a conductivity bridge TDS model 72 at Domiat University, Egypt. IR spectra were recorded on an 800-PC FTIR Shimadzu spectrophotometer using KBr pellets ($4000\text{-}400\text{ cm}^{-1}$) at Cairo University. Far-IR spectra were recorded using spectrophotometer model 6300 FTIR in the Egyptian Petroleum Research Institute. Magnetic moments were determined using a Sherwood balance at room temperature (25°C) with $\text{Hg}[\text{Co}(\text{NSC})_4]$ as a calibrant at Mansoura University. Diamagnetic corrections for *L*-lysine.2HCl and the metal atoms were computed using Pascal's constants^[15]. Electronic spectra of the complexes in Nujol mulls were recorded on a Unicam UV2 spectrophotometer at Mansoura University.

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Synthesis of ligands and metal complexes

The synthesis of the metal complexes with the general formula, $R_2[MCl_4]$, were obtained by grinding equivalent amounts of the solid metal salts under investigation with *L*-lysine. $2HCl$ by tribochemical reaction. The mixtures were mixed and grinded in agate mortar at room temperature till the reactants become in fine powder. The complexes were dried in an oven at 60 °C and kept in desiccators over $CaCl_2$.

Biological activity

Cytotoxicity assay

Evaluation of the cytotoxicity of the prepared complexes against HCT-116 (colon) and MCF-7 (breast) cell lines was carried out in the Regional Center for Mycology and Biotechnology (Al-Azhar University). Doxorubicin and Vinblastine were used as standard drugs. The cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well

in 100 μ L of growth medium. Fresh medium containing different concentration of the test sample was added after 24h of seeding, serial two-fold dilution of tested chemical compound were added to confluent cell monolayer dispensed into 96-well flat-bottomed microtiter plates (Falcon, NJ, USA) using multichannel pipette. The microtiter plates were incubated at 37°C in humidified incubator with 5% CO_2 for a period of 48h. Different concentrations of the sample (50, 25, 12.5, 6.25, 3.125 and 1.56 μ g) were added and the incubation was continued for 48h and a viable cell yield was determined by a colorimetric method, after end of the incubation period, medium were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly and then the absorbance of the plates was measured after gently shaken on microplate reader^[16]. The relationship between the surviving fraction and

TABLE 1 : Elemental analyses and some physical data of the metal complexes

Complex; chemical formula	M.p., °C	λ_{max} .	Λ_m (DMSO; $ohm^{-1}cm^2$ mol^{-1})	U_{eff} (B.M)	C% F(Calc.)	H% F(Calc.)	N% F(Calc.)	M% F(Calc.)
$[NH_2-HC-COOH-(CH_2)_4-NH_3]_2[CuCl_4]$; $C_{12}H_{30}N_4O_4CuCl_4(1)$	180	304.00 380.00 718.00	----	2.06	30.0 (29.8)	6.1 (6.1)	11.3 (11.2)	12.7 (12.7)
$[NH_2-HC-COOH-(CH_2)_4-NH_3]_2[BaCl_4]$; $C_{12}H_{30}N_4O_4BaCl_4(2)$	257	248.00 288.00 306.00 416.00	115	Diamag	24.4 (25.1)	4.6 (5.3)	9.3 (9.8)	24.4 (23.9)
$[NH_2-HC-COOH-(CH_2)_4-NH_3]_2[CdCl_4] \cdot 2H_2O$; $C_{12}H_{30}N_4O_4CdCl_4(3)$	234	254.00 384.00 414.00 564.00	----	Diamag	23.9 (24.7)	5.6 (5.9)	9.5 (9.6)	19.7 (19.2)
$[NH_2-HC-COOH-(CH_2)_4-NH_3]_2[PdCl_4]$; $C_{12}H_{30}N_4O_4PdCl_4(4)$	200	238.00 358.00 400.00 420.00 420.00	115	Diamag	27.4 (26.6)	5.5 (5.6)	10.3 (10.3)	19.7 (19.6)

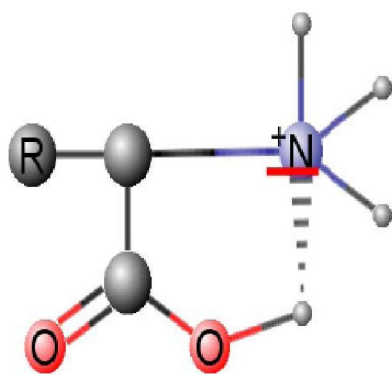


Figure 1 : Hydrogen bond in lysine.2HCl

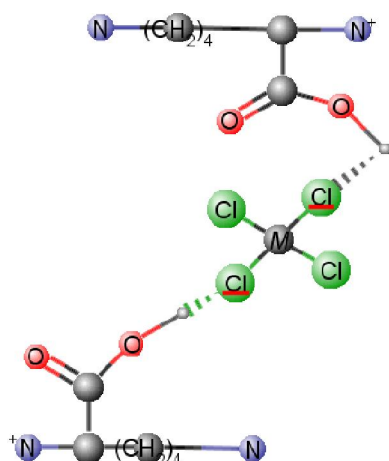


Figure 3 : The structure of the complexes with the formula $R_2[MCl_4]$

drug concentration was plotted to get the survival curve of each tumor cell line after specific compound was added. Inhibition of cell proliferation (IC_{50}) for test compounds (A, B and D) ($\mu\text{g sample/L}$) were recorded. The IC_{50} is the concentration of treatment required to induce 50% inhibition of cell growth^[16].

RESULTS AND DISCUSSION

The analytical data of the complexes are listed in TABLE 1. Comparison of the elemental analyses for the calculated and found percentage indicates that the compositions of the complexes coincide with proposed formulae. All the complexes are decomposed in water but freely soluble in DMF and DMSO. The decomposition of complexes in H_2O is mainly due to the weak hydrogen bond formed within the complex. The values of molar conductance in DMSO TABLE 1 suggest that all the complexes are

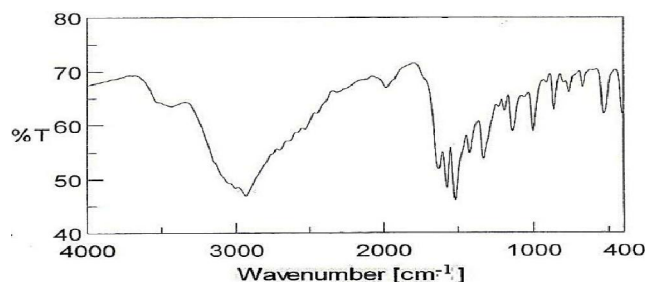


Figure 2 : IR spectrum *L*-lysinetetrachloropalladate(II) in KBr

electrolytic in nature^[17]. The melting points of the solid complexes TABLE 1 lie in the 180-257 °C range, suggesting that the strength of the bond between *L*-lysine.2HCl and the metal ions are quite stable.

IR spectra of the complexes derived from *L*-lysine.2HCl

The IR spectrum of the free ligand (*L*-lysine.2HCl) shows the absence of any bands in the 3400-3100 cm^{-1} regions. This suggests that the two NH_2 groups of *L*-lysine.2HCl are changed to NH_3^+ as well as the absence of H_2O molecules in the compound as shown in Figure 1. Several bands are observed at 3024, 2814, 1725, 1620 and 1571 cm^{-1} . The first two strong bands are mainly due to the strong hydrogen bond between the OH and NH_3 ($\text{N}\dots\text{H}-\text{O}$) vibration. The latter two bands at 1620, and 1571 cm^{-1} are attributed to the $\nu_{\text{as}}(\text{COO})$ and $\nu_{\text{s}}(\text{COO})$ vibrations^[18-22], respectively. The IR spectrum of the *L*-lysinetetrachloropalladate(II) in KBr complex is recorded in Figure 2.

The most important bands in the metal complexes, $[\text{NH}_3-\text{CH}(\text{COOH})-(\text{CH}_2)_4-\text{NH}_2]_2[\text{CuCl}_4]$ (1), $[\text{NH}_3-\text{CH}(\text{COOH})-(\text{CH}_2)_4-\text{NH}_2]_2[\text{BaCl}_4]$ (2), $[\text{NH}_3-\text{CH}(\text{COOH})-(\text{CH}_2)_4-\text{NH}_2]_2[\text{CdCl}_4]$ (3), $[\text{NH}_3-\text{CH}(\text{COOH})-(\text{CH}_2)_4-\text{NH}_2]_2[\text{PdCl}_4]$ (4), are observed in the regions 3493-3409, 3234-3002, 2927-2900, 1735-1681 and 1680-1587 cm^{-1} . The observation of the bands in the 3493-3409 and 3234-3002 regions assigned to NH_2 vibration indicating that one of the NH_3^+ group in the free ligand is changed to NH_2 on complex formation. These bands are assigned to $\nu_{\text{as}}(\text{NH}_2)$ and $\nu_{\text{s}}(\text{NH}_2)$ vibrations, respectively. The band observed in the 2927-2900 regions is $\nu(\text{CH})$ vibration. The last two bands are assigned to $\nu_{\text{as}}(\text{COO})$ and $\nu_{\text{s}}(\text{COO})$ vibrations^[20-22], re-

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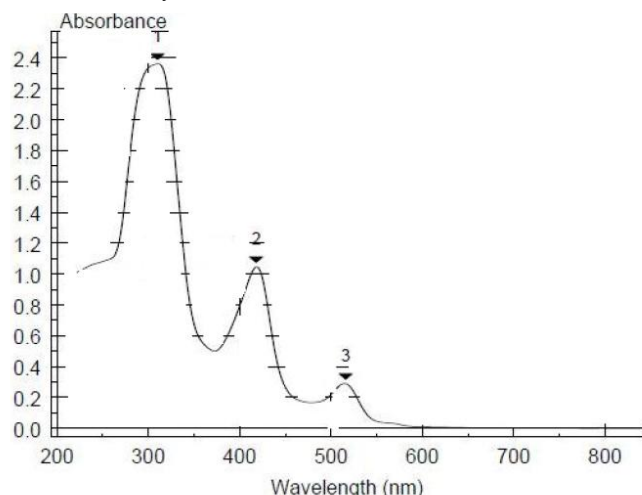


Figure 4 : The electronic spectrum of the Cu^{2+} complex spectively. Also, the band observed at 1585 cm^{-1} is assigned to NH_3^+ group suggests that the ligand is mainly existed in the form of Zwitterion. Also, the data obtained suggest that the ligand is bonded to the metal ions forming a complex of the type $[\text{L}]^{2+}[\text{MX}_4]^{2-}$. Finally, the bands observed in all complexes in the $1900\text{--}2000$ and $2600\text{--}2500\text{ cm}^{-1}$ region suggests the presence of $\text{O-H}\cdots\text{Cl}$ hydrogen bond^[23] as shown in Figure 3.

Electronic spectra and magnetic measurements

The electronic spectrum of the Cu^{2+} complex is shown in Figure 4. All the complexes show two main bands in the $272\text{--}418$ and $374\text{--}516$ nm regions which are assigned to $\pi\rightarrow\pi^*(\text{COOH})$ and $n\rightarrow\pi^*(\text{COOH})$ transitions^[24], respectively. The Cu^{2+} complex exhibits three bands at 310 , 385 and 520 nm. The first two bands are due to charge-transfer while the third band is due to d-d transition. The observation of these bands suggests that the Cu^{2+} complex has a distorted-tetrahedral geometry around the Cu^{2+} ion^[25]. The value of magnetic moment for the Cu^{2+} complex (2.06 BM) suggests the absence of Cu-Cu interactions.

Far-IR spectra of the metal complexes

The Far-IR spectrum ($600\text{--}50\text{ cm}^{-1}$) of the tetrahalopalladate(II) complex of the general formula, $\text{L}_2[\text{PdCl}_4]$, is recorded in Figure 5. The spectra in the complexes show two bands in the $265\text{--}295$ and $54\text{--}84\text{ cm}^{-1}$ regions assigned to $\nu(\text{M-Cl})$ stretching^[26] and $\nu(\text{Cl-M-Cl})$ bending, respectively.

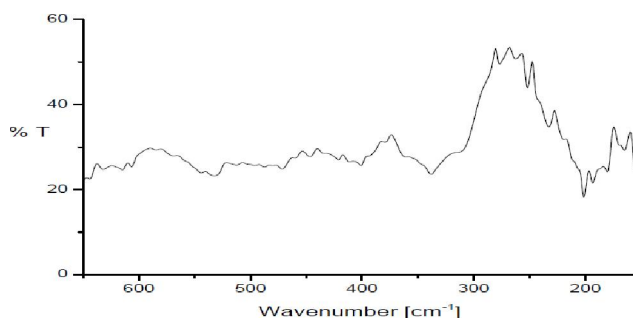


Figure 5 : Far-IR spectrum of $\text{L}_2[\text{PdCl}_4]$

Evaluation of cytotoxic activity on human tumor cell lines

Activity against breast cancer cell lines (MCF-7)

The data illustrate that the order of the activity of the complexes against breast cancer is in the order: $3 > 1 > 4 > 2$ with IC_{50} values of (0.47 , 6.0 , 34.3 and $37.0\text{ }\mu\text{g/mL}$). Doxorubicin (DOX) a drug with antineoplastic activity was used in this study as standard drug, since it is widely used in the treatment of tumor cells. Bis-*L*-lysine tetrachlorocadmate (3) is promising to inhibit the growth of breast cancer lines while bis-*L*-lysine tetrachloropalladate complex (4) shows the least antitumor activity due to its low solubility.

Activity against cervical cancer

All tested compounds are effective against HELA (Cervical cancer) illustrate that the order of their activities is $3 > 1 > 4 > 2$. Bis-*L*-lysine tetrachlorocadmate (3) and bis-*L*-lysine chlorocuprate (1) are very successful to inhibit the growth of the cervical cancer. The bis-*L*-lysine tetrachlorobarimate (2) has the worst effect.

Activity against colon cancer

The activity of tested compounds against HCT (colon cancer) illustrates that the order of their activities is in the order: $3 > 1 > 2 > 4$. Bis-*L*-lysine tetrachlorocadmate (1) is very potential agent to inhibit the growth of the colon cancer followed by bis-*L*-lysine diiododichlorocadmate (I_2) and bis-*L*-lysine tetrachloropalladate (4) has the lowest potent activity. Cytotoxic activities of these complexes are mainly due to their differential solubility.

TABLE 2 : Inhibition of cell proliferation (IC₅₀ µg sample/L) for complexes

Complex	Breast cancer	Cervical cancer	Colon cancer	Larynx cancer	Hepatocellular cancer
Bis- <i>L</i> -lysinetetrachlorocuprate(II)(1)	6.0	9.3	6.9	9.7	10.9
Bis- <i>L</i> -lysinetetrachlorobarimate(II)(2)	37.0	37.6	36.6	40.0	32.1
Bis- <i>L</i> -lysinetetrachlorocadmiate(II)dihydrate(3)	0.47	0.47	0.73	0.51	0.69
Bis- <i>L</i> -lysinetetrachloropalladate(II)(4)	34.3	16.5	47.8	40.0	21.8

Activity against larynx cancer

The activity of compounds under investigation against HEP2 (Larynx cancer) shows that the order of their activities is as follow: 3>1>2≈4.

Both of bis-*L*-lysinetetrachlorocadmiate(3) and bis-*L*-lysinetetrachloropalladate (1) are the most potent while bis-*L*-lysinetetrachlorobarimate (2) and bis-*L*-lysinetetrachloropalladate(4) are the least effective in activity against HEP2 (Larynx carcinoma).

Activity against hepatocellular cancer

The activities of compound against HEPG2 (Hepatocellular cancer) illustrate that the order of their activities is as follow: 3>1>4>2. Bis-*L*-lysinetetrachlorocadmiate (3), and bis-*L*-lysinetetrachlorocuprate(1) are very successful to inhibit the growth of the hepatocellular cancer. Both the bis-*L*-lysinetetrachloropalladate (4) and bis-*L*-lysinetetrachlorobarimate (2) show less activity. All the data are recorded in TABLE 2.

Cadmium and copper complexes(3 and 1) are the most active compounds against all tumor cell lines. The results suggest that the positively charged polar head of the complexes provides the basis for its anticancer specificity, whereas the amino acid tail may aid in its insertion into the plasma membrane altering its mosaic structure in response to the negative trans-membrane potential. It should be also noted that effects of these complexes are found to be dependent on the type of the tested tumor cell line. Results illustrated that cadmium complex shows excellent cytotoxic activity, which can be attributed to the ability of cadmium complex to produce non-covalently interact with DNA double helix rather than forming coordinated covalent adducts with DNA. The non-covalent DNA interactions included intercalative, electrostatic and groove binding of

metal complexes along the major or minor DNA groove. In most cases, the metal acted as an inorganic modifier of the organic backbone of the bioactive molecule and ligands granted DNA affinity and specificity^[27].

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