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Original article

Synthesis, structure, electrochemistry and spectral characterization of (D-glucopyranose)-4-phenylthiosemicarbazide metal complexes and their antitumor activity against Ehrlich Ascites Carcinoma in Swiss albino mice☆

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ABSTRACT

The novel glycosyl saccharide derivative, (D-glucopyranose)-4-phenylthiosemicarbazide (LH) and its complexes, with cobalt(II), nickel(II), copper(II) and zinc(II) were synthesized, characterised and tested for cytotoxic effects. The copper complex, [CuLCl] inhibited Ehrlich Ascites Carcinoma (EAC) induced cancer cell lines in Swiss albino mice at $LC_{50} = 1.94 \times 10^{-8}$ ($LC_{50} = 2.76 \times 10^{-8}$ for cisplatin) and so distinctly better than free ligand and other complexes.

1. Introduction

Biosynthesis of carbohydrates is a basic process of life and quantitatively the most important one. Monosaccharides are fundamental biomolecules in that they are the building blocks of polysaccharides. They are the constitutional parts of complex lipids (glycolipids) and proteins (glycoproteins). They are also building blocks of nucleotides and, hence, of nucleic acids and of the chemical ADP/ATP energy storage system. Carbohydrates are of primary importance as energy sources for living organisms. Due to the properties inherent to this class of molecules, carbohydrates have been utilized to prepare bioactive materials [1], better-targeted drugs [2], as well as for the functionalization of hydrophobic materials [3]. Metal–carbohydrate interactions are also of significant interest in bioinorganic chemistry [4–6]. Thus, carbohydrates exert a wide range of functions in living organisms, and due to the wide distribution of metals and their complex functions for all forms of life, metal–carbohydrate interactions are a key for understanding bioinorganic chemistry, and the study of complexation of carbohydrates to metals is one of the main objectives of carbohydrate coordination chemistry.

Present investigation focuses on the design and synthesis of new saccharide derivative type of chelators by the introduction of thiosemicarbazide as anchoring group, which can provide well-defined binding environment as well as increase the stability of the resultant metal complexes over the simple saccharide complexes itself. In present study the anchoring groups introduced via *N*-glycosylic linkage at the anomeric carbon at C-1 position of the saccharide moiety. Thiosemicarbazide in the present investigation are used hoping that the toxophoric functional group –C═S will be away from the coordinating site so that these functional groups could provide 'point of attachment', a system which mimics certain classes of biological systems and in addition such groups could be toxic to microbes when used as drugs. A potential benefit of utilizing this approach is that the carbohydrate can remain pendant, thereby being freely available to interact with carbohydrate transport and metabolic pathways in the body. From these metal-binding properties of D-glucose, it is hoped that such would aid in the understanding of the structural chemistry of metal ions interacting with saccharides, as an actual biological system, and thereby in the interpretation of some particular biological processes.

☆ Dedicated in memory of Late Dr (Mrs) Geeta M. Kulkarni (1959–2009).

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2. Results and discussion

2.1. Chemistry

4-Phenylthiosemicarbazide [7] was prepared following the literature procedure and D-glucose was purchased from Qualigens. The free saccharide was treated with 4-phenylthiosemicarbazide in refluxing methanol in presence of a small amount of NH_4Cl as shown in Scheme 1, to get (D-glucopyranose)-4-phenylthiosemicarbazide (LH). Cobalt(II), Nickel(II), Copper(II) and Zinc(II) complexes of the ligand were prepared. The reaction sequences are outlined in Scheme 2. The characterization of the ligand and complexes cover both in the solution as well as in the solid state using a variety of analytical and spectral techniques. The complexes are mononuclear with M:L ratio being 1:1. All the complexes were found to be soluble in ethanol, methanol, chloroform, acetone, dimethylsulphoxide, dimethylformamide and were found to be non-electrolytic in nature. The growth of single crystals of these complexes for X-ray studies is very difficult owing to their amorphous nature and we were unsuccessful in our attempts to do so. The composition and coordination geometry of these complexes have been established on the following experimental observations. The molar conductance values in dimethylsulphoxide fall in the expected range (20–31 mho cm^2mol^{-1}) of non-electrolytes [8] indicating that chloride ions are inside the coordination sphere. The complexes were analyzed for metal, carbon, hydrogen, nitrogen, sulfur and chloride. The analytical data, conductivity and magnetic moment of the complexes are given along with their synthetic procedures.

2.2. FTIR spectra

Formation of the N-glycoside was observed by comparing the FTIR spectrum of the ligand, with the spectra of the saccharide, D-glucopyranose and the corresponding 4-phenylthiosemicarbazide. When 4-phenylthiosemicarbazide reacts with the saccharide, the band corresponding to the primary amine was absent, and that of the secondary amine was shifted to higher frequency in the ligand due to the formation of the *N*-glycoside. The spectra of complexes are indicative of the cleavage of extensive intermolecular hydrogen bonds existing in the solid state of the parent ligand [9,10], thus resulting in a broad band $3410 \pm 10\ cm^{-1}$ region with a low frequency component as a shoulder at $3290 \pm 10\ cm^{-1}$ upon complex formation. The sharp bands at 3335 and 3444 cm^{-1} were observed in the copper complex due to secondary amine and hydroxy groups of the ligand moiety. Broad band in the remaining complexes in the region 3413–3550 cm^{-1} clearly demonstrate the presence of aqua molecules. Coordinated mode of aqua molecules in case of nickel complex and lattice held water molecules in case of cobalt and zinc complexes were confirmed by their thermal studies. Ligand and all the complexes show a prominent band in the region 1120–1080 cm^{-1} due to C=S absorption, which is shifted to lower frequency side after complexation, indicating the coordination of sulphur atom. The band observed between 2920 and 2930 cm^{-1} are assigned to νCH indicating the presence of saccharide moieties in the product. The sharp absorption band in the region 1640–1590 cm^{-1} in the ligand and complexes is assigned to NH deformation vibration. Absorption bands corresponding to the anomeric properties of the *N*-glycosyl amines were observed in the spectra. The strong bands observed in all the complexes viz., 753–751 and 695–690 cm^{-1}, indicate the presence of both α and β anomers [10], which is in consistent with the observations made by NMR studies.

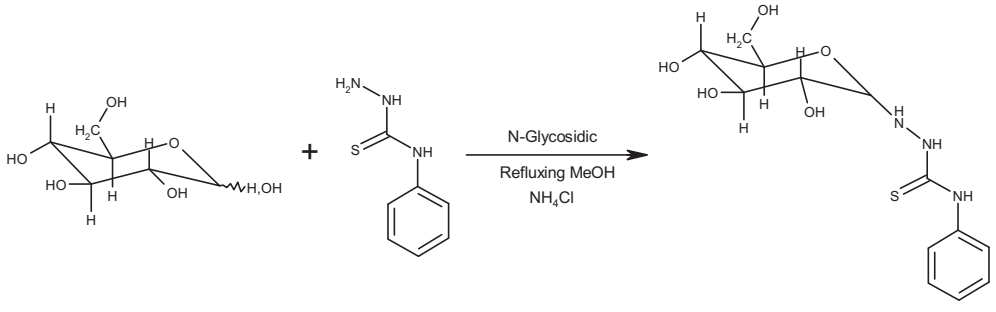
2.3. ^1H NMR spectra

The ^1H NMR spectra of the ligand (LH) and the zinc complex were recorded in DMSO-d_6 and the assignments were made by relative comparison. The comparison clearly demonstrates the formation of metal-saccharide complexes.

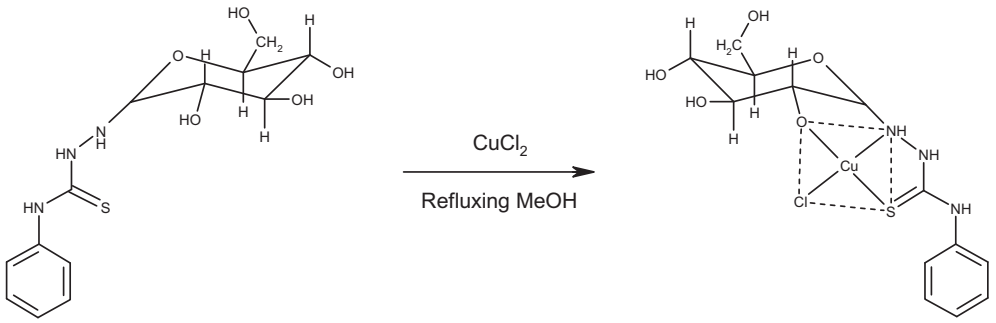
As the glycosylation occurs at C-1 centre by condensation of the saccharide with the 4-phenylthiosemicarbazide, the spectrum of the *N*-glycoside was devoid of C-1-OH resonance which otherwise present in the corresponding saccharide spectrum around 6.49–6.78 ppm. Signals corresponding to the OH and CH groups of the saccharide moiety were identified in the region 2.0–3.7 ppm from the spectrum of (D-glucopyranose)-4-phenylthiosemicarbazide. The down field shift in the glycosylic –NH proton was observed in the zinc complex (9.2–9.8 ppm) compared with the ligand (observed at 8.9 ppm). The observed downfield shift of the glycosylic N–H in the metal ion complex indicates the coordination of glycosylic nitrogen to the metal centre. It is obvious from the comparison of the chemical shift values of saccharide–OH group of the ligand with corresponding zinc complex shows that 2–OH group could have disappeared as this is involved in the formation of a five membered chelate with the metal ion after deprotonation. However, in the complex it is very difficult to trace out the number of –OH groups [10,11]. In the ligand and zinc complex, the C–1–H resonance appears as doublet at 4.99 ppm corresponding to the presence of both α and β anomers. Correspondingly, two different signals were observed for the C-1-NH (glycosyl) around 9.2–9.8 ppm indicating the presence of mixtures of anomers [11].

2.4. Electronic spectra

The electronic spectra were recorded in DMSO solution and data are given in the Table 1. The observation made by comparing the infrared spectrum of the ligand with those of metal complexes it was observed that the substantial energetic and charge distributional changes caused by complex formation. Hence it could be presumed that such changes also appear in the spectra of complexes in the ultra-violet and visible frequency range. The



Scheme 1. Synthesis of (D-glucopyranose)-phenylthiosemicarbazide LH.



Scheme 2. Synthesis of [CuLCl].

ligand exhibits absorption bands in UV-visible region around 274, 320 and 375 nm. The first band below 300 nm is assigned to a ligand $\pi \rightarrow \pi^*$ transition. The band around 320 nm is assigned to $n \rightarrow \pi^*$ transition associated with imine function of thiosemicarbazide. Another band observed at 375 nm is assigned to $n \rightarrow \pi^*$ transition originating from the thioamide function [12] of thiosemicarbazide. Comparison of the absorption spectrum of the ligand with that of the metal complexes indicated the complex formation. The band around 370 nm in ligand shifted to lower wavelength by 20–30 nm in the metal complexes. In addition to this, the metal complexes, except zinc complex, exhibit another band in the range 415–474 nm is characteristic of d-d transitions. However, the ratio of the intensities of all the bands slightly varies in complexes [11]. Such intensity ratio analysis would be used in establishing the binding of metal ions with the saccharide based polymers and antibiotics.

2.5. Magnetic moment studies

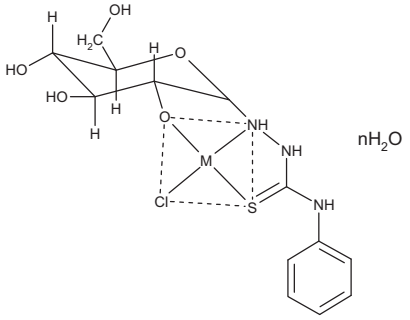
The magnetic moment value for the cobalt(II) complex is found to be 4.5 BM, which lie within the expected range for four-coordinated tetrahedral cobalt(II) complexes. The observed room temperature magnetic moment of Ni(II) complex is found to be 2.96 BM, which is consistent with high-spin octahedral geometry [13]. The slightly higher than spin-only value (2.83 BM) of observed moment may be due to mixing of upper states via spin orbit coupling [14]. For the copper (II) complex, magnetic moment value was found to be 1.93 BM, which lie appreciably above the spin-only value of 1.73 BM for Cu(II) ion, indicating the presence of single unpaired electron and suggesting the square planar structure for the complex [15].

2.6. Circular dichroism studies (CD)

CD spectra of ligand and all the complexes were recorded in DMSO in the region of 200–900 nm. Though the Cotton effect is not prominent in ligand, they show a characteristic (+) curve in the region 280–300 nm indicating the dextrorotatory nature having the 'S' configuration at the C-2 carbon [16]. The CD spectra of complexes showed conspicuous Cotton effects in the d–d transition region and strongly indicated the coordination of sugar moieties to the metal center. Further the bimodal shape of peaks in complexes with (−)ve peak (negative Cotton effect) in the region 600–610 nm and corresponding (+)ve peak (positive Cotton effect) in the region 700–750 nm and the observation of negative CD couplet in the region 300 nm running into vacuum UV (VUV) region indicates an "exiton coupling" [17] probably arising due to a rapid equilibrium in which both the anomeric form of the sugar moieties interact with the metal center alternatively. Almost same CD pattern was observed for all the complexes. The spectral results indicate similarities in the configuration around the metal centre in these complexes. The study confirms that there seems to exist certain preferential orientations for particular saccharides in their binding to metal ions Fig. 1.

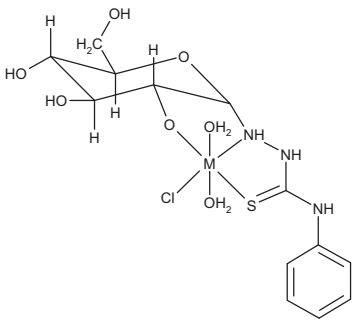
2.7. EPR study

The solid state EPR spectrum of the mononuclear copper(II) complex was recorded in the X-band region at room temperature. The EPR spectrum of the mononuclear copper(II) complex shows two lines having $g_{||}$ value at 2.11 and $g_{\perp}$ at 2.03. $g_{||}$ value being less than 2.3, witnesses more covalency in metal-ligand interaction [18]. Further it is noteworthy that the relation $g_{||} > g_{\perp} > g_e$ (2.0027) is typical of axially symmetric d^9 Cu^{II} ion having one unpaired electron in $d_{x^2-y^2}$ orbital [19].



M = Co(II), Cu(II) and Zn(II)

n = 2 for Co(II) and nil for Cu(II) and Zn(II)



M = Ni(II)

Table 1
Electronic spectral data of the compounds.

Compound	λ_{max} (nm)
LH	274, 320, 375
[CoLCl]·2H$_2$O	273, 327, 373, 619
[NiLCl·2H$_2$O]	270, 300, 373, 474
[CuLCl]	260, 327, 340, 421
[ZnLCl]	268, 373, 378

2.8. FAB mass and thermal studies

The analyses of FAB mass spectra, without any ambiguity, conclude the monomeric nature for all the complexes, which parallels the results of elemental analyses. The FAB mass spectrum of copper(II) complex (as a representative) shows the molecular ion peak at *m*/*z* 427. The spectrum also shows some prominent peaks due to molecular cations, various fragments of the complexes are due to isotopic atoms like copper and chloride ions (Fig. 2).

Room temperature stability for all the complexes was revealed by their thermogravimetric analysis. All the complexes show a similar pattern of decomposition at higher temperature. All the complexes except cobalt and nickel complexes show an initial weight loss of 7.2% in the temperature range 240–270 °C, which corresponds to loss of coordinated chloride ion. On increasing the temperature further, weight loss was observed up to 510 °C. The observed weight loss is attributed to the loss of ligand moiety in all the complexes. No weight loss was observed beyond 510 °C, possibly due to the formation of stable metal oxides. The observation at the same temperature range for nickel complex shows a weight loss of 16.2%, which corresponds to combined loss of coordinated chloride ion along with two molecules of coordinated water. In case of cobalt complex, the initial weight loss of 8.01% in the temperature range 100–110 °C corresponding to the loss of lattice held water molecules. These observations further supports the composition of Ni(II) and Co(II) complexes, which is fixed as a result of elemental and FAB mass studies. The decomposition pattern at these temperature range rules out the possibility of lattice held water molecules (Fig. 3).

2.9. Electrochemical studies

The redox activity of ligand and all the complexes were studied in the range +0.2 to −0.8 V versus the ferrocene-ferrocenium redox system in DMSO (0.1 M tetrabutylammoniumperchlorate as supporting electrolyte). No peaks corresponding to the oxidation/reduction have been observed in the working potential range, as the ligand was shown to be electrochemically inactive. The redox properties of all the complexes were explored by cyclic voltammetry. The copper and cobalt complexes are shown to be redox active. The voltammogram (Fig. 4) data are summarized in Table 2. The copper complex undergoes one step one-electron reduction process ($Cu^{II} \rightarrow Cu^{I}$) in forward scan and one step one-electron oxidation ($Cu^{I} \rightarrow Cu^{II}$) process in reverse scan. The electrochemical process is found to be quasi-reversible, as evidenced by (i) the peak-to-peak separation ΔE_p is greater than 59 mV, (ii) the current ratios i_{pa}/i_{pc} is less than 1, and (iii) E_{pc} shifts negatively with increasing scan rate [20]. Cobalt complex exhibits a two one-electron quasi-reversible oxidation processes ($Co^{II} \rightarrow Co^{III}$, $Co^{III} \rightarrow Co^{IV}$), when scanned in anodic direction, two cathodic peaks had followed respective cathodic peak in reverse scan ($Co^{IV} \rightarrow Co^{III}$, $Co^{III} \rightarrow Co^{II}$). The structural reorganizations accompanying a redox change should affect essentially the kinetics of the heterogeneous electron-transfer process. According to the Marcus theory, inner-sphere rearrangements, enhancing the activation barrier of the electron-transfer, slow down the rate of the process, so causing a departure from electrochemical reversibility [21]. In the present series of complexes the largest rearrangement is likely experienced by the planar copper complex.

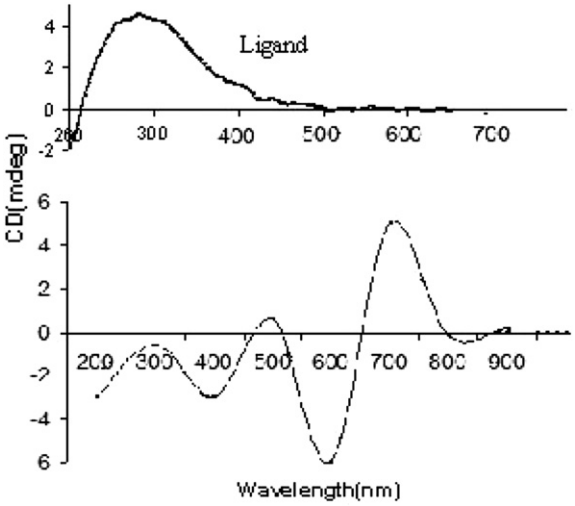


Fig. 1. CD spectra of Ligand and [CuLCl].

3. Biochemistry

3.1. Antitumor activity of complexes against Ehrlich Ascites Carcinoma in Swiss Albino mice

The compounds were tested using the short term *in vitro* cytotoxicity towards EAC (Ehrlich Ascites Carcinoma) cells as a preliminary screening technique of tryphan blue exclusion method (Cell Viability Test) for their cytotoxic potential [22]. Results of the short term *in vitro* cytotoxicity of the compounds are shown in Table 3. These preliminary experiments were carried out mainly with five different concentrations of the compounds. All the compounds were found to be cytotoxic and produced 50% cell death at a concentration of 19.1 μg/ml. At 50 μg/ml concentration the standard (Cisplatin) showed 96% cell death. At 50 μg/ml concentration the copper(II) complex showed more than 85% cell death while ligand and other complexes showed ∼70% cell death at 50 μg/ml concentration. All the compounds were found to have considerable cytotoxicity in the cell viability test.

The tumor inoculated control animals gained a substantial weight by day-0. They gained a maximum weight of 19% by day-15. Cisplatin administration (on 10th post inoculation day) significantly ($p < 0.05$) reduced weight gain as compared to control on day-15. The compounds significantly ($p < 0.05$) reduced the weight gain on day-15 as compared to control (Table 4).

The effect of compounds on survival of tumor bearing mice is shown in Table 5. Cisplatin significantly prolonged the mean survival time ($p < 0.05$) with respect to its control. It shows a significant increase in the percentage life span of animals (ILS > 50). On the other hand, all the compounds significantly prolonged the mean survival time. The influence of all the compounds on %ILS was more than 25%. By convention, a 25% increase in life span is considered as possible anticancer activity of a test compound [23].

4. Conclusion

The present study demonstrates the binding nature of the newly synthesized unprotected N-glycosyl saccharide derived from

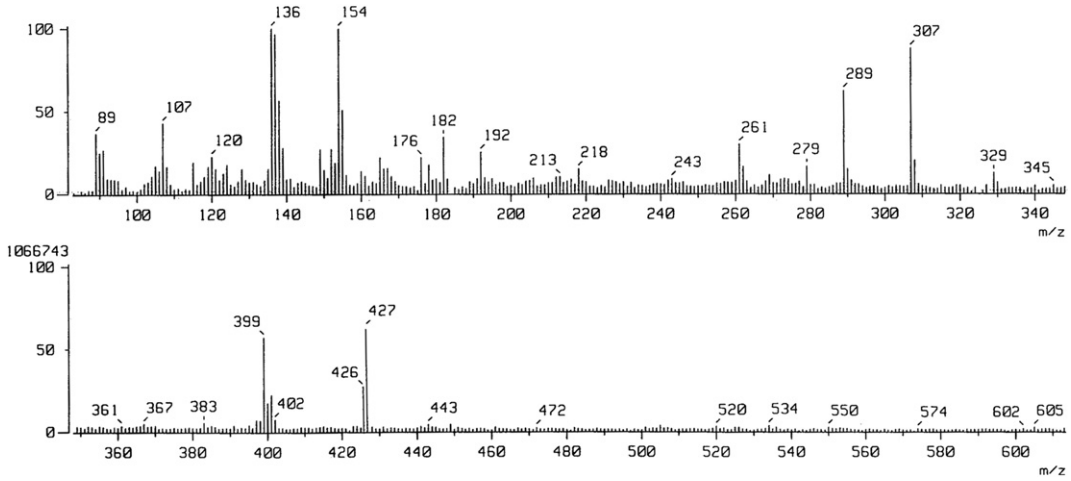


Fig. 2. FAB mass spectrum of [CuLCl].

4-phenylthiosemicarbazide, with the Co(II), Ni(II), Cu(II) and Zn(II) chlorides. Elemental analysis and molar conductance studies reveal that, the complexes are mononuclear and are non-electrolytes. All the trends observed with the ^{1}H NMR chemical shifts and CD measurements together indicate that the ligand is found to be a mixture of both α and β anomers. The specific rotation of the ligand is positive (dextrorotatory), which is consistent with their corresponding anomeric assignments. The study of complexation of ligand with different metal ions show binding of the glycosylic-NH, C-2-OH group from one of the saccharide residue and –C═S group of the thiosemicarbazide unit as revealed by the FTIR and NMR studies. Therefore the ligand acts as monobasic, tridentate 'SNO' donor. All the above observations together allow structural motif to be proposed for the binding of metal ions to the ligand. Although M(II)-saccharide complexes exist at physiological pH, the complexation is favored under alkaline conditions. From these metal-binding properties of D-glucose, it is hoped that such would aid in the understanding of the structural chemistry of metal ions interacting with saccharides, as an actual biological system, and thereby in the interpretation of some particular biological processes.

The compounds showed considerable cytotoxic activity in the tryphan blue exclusion method. In the *in vivo* cancer model, the compounds significantly ($p < 0.05$) reversed the tumor induced changes in the parameters monitored, viz., percentage increase in body weight, percentage increase in life span, tumor viable count and haematological parameters (total and DLC of WBC, total RBC and Hb). These effects were almost comparable to cisplatin. The compounds however were found to have good effect in prolonging the life span as compared to standard. These findings imply that the compounds might be having some anticancer principles. Based on the data of the present study, it is very difficult to suggest the possible mechanism for the anticancer effects. The compounds tested in present study have shown promising cytotoxic activity when screened using the *in vitro* method and at the same time were shown to have good activity when tested using the Ehrlich Ascites Carcinoma model. Though it is very difficult to conclude anything at this stage, it can be assumed that after testing against various other

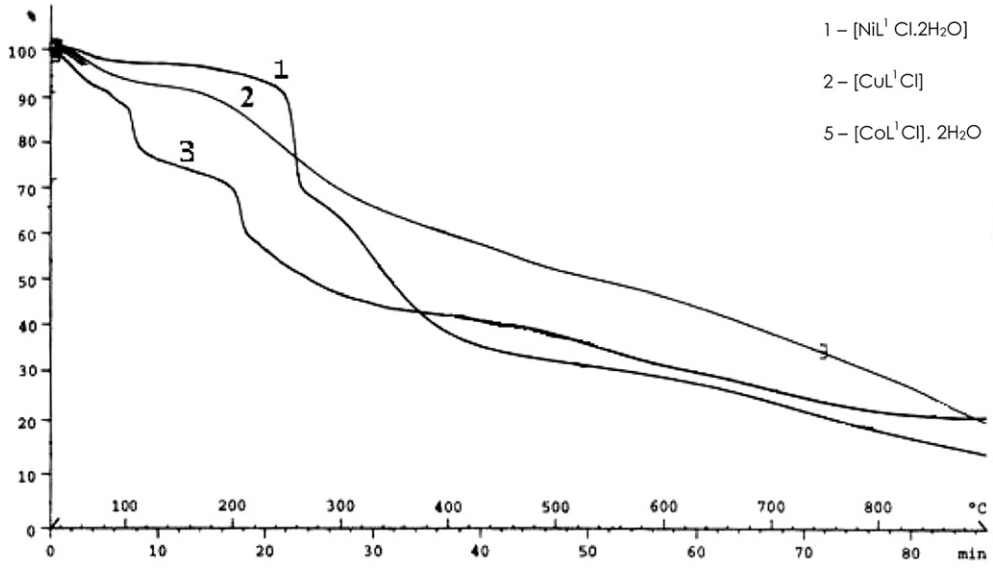


Fig. 3. Thermograms of complexes.

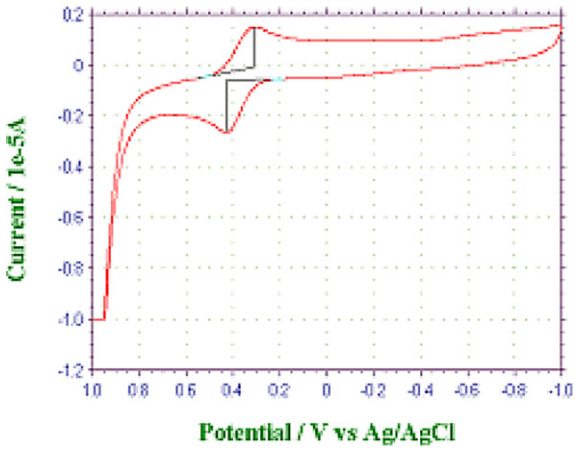


Fig. 4. Cyclic voltammogram of [CuLCl].

cancer models and at different doses these compounds may prove to be safer drugs for tomorrow.

5. Experimental protocols

All chemicals used were of reagent grade. Solvents were distilled prior to use in the synthetic part. The metal content of the complexes was estimated by using standard methods. All the compounds were analyzed for carbon, hydrogen, nitrogen and sulfur by Thermo quest elemental analyzer at STIC Cochin University of Science and Technology, Cochin. Magnetic susceptibility of complexes was measured at room temperature on a Faraday balance using $Hg[Co(SCN)_4]$ as a calibrant. Electronic spectra were recorded using Varian Cary 50 Bio UV-visible spectrophotometer in DMSO. The IR spectra of ligand and its complexes were recorded as KBr pellets in the region 4000–400 cm^{-1} on Nicolet 170 S × FT-IR spectrometer. Proton magnetic resonance spectra were recorded on a Bruker 300 M Hz spectrometer in DMSO-d_6 using TMS as an internal standard. The EPR spectrum of copper(II) complex was recorded at both room on Varian E-4 X-band spectrometer using TCNE as g-marker. Conductivity measurements were measured at 10^{-3} M solutions of complexes in DMSO using ELICO-CM82 conductivity bridge provided with a cell having cell constant 0.51. CD measurements of saccharide ligand and their complexes were made on a JASCO-J-715 spectropolarimeter. The cyclic voltammetric experiments were carried out with a three electrode apparatus using a CHI1110A electrochemical analyzer (USA). Cyclic voltammetric data were recorded using a glassy carbon working electrode (0.082 cm^2), a platinum counter electrode, and an Ag/Ag+ reference electrode. Glassy carbon electrode surfaces were polished with 0.05 mm alumina, rinsed in water, and air-dried immediately before use. The electrochemical experiments were carried out and the positions of the waves were compared to the potential of the ferrocene/ferrocenium couple. The DMSO solution (containing 0.1 M tetrabutylammoniumperchlorate, as supporting electrolyte, 10^{-3} molar concentration of the ligand and each of the complexes) was placed in a single-compartment electrochemical cell and degassed by bubbling with N_2(g) saturated with DMSO. A N_2 atmosphere was continuously maintained above the solution while the experiments were in progress. TG and DTA measurements of the complexes were recorded in nitrogen atmosphere on Universal V2.4F TA Instrument keeping final temperature at 800° C and heating rate was 10° C/min. The FAB mass spectra were drawn from JEOL SX 102/DA-6000 mass spectrometer using Argon/Xenon (6 kV, 10mA) as the FAB gas.

Table 2
Cyclic voltammogram data for the complexes.

Complex	$E_{1/2}$/mV (ΔE_p/mV)
[CuLCl]	399(143)
$[CoLCl]\cdot 2H_2O$	159(222), 149(199)

Table 3
Short term in vitro cytotoxicity of compounds towards EAC cells.

Compounds	Percentage cell death at different concentrations after 3 h					LC_{50} in $M \times 10^{-8}$
	1 μg/ml	5 μg/ml	10 μg/ml	25 μg/ml	50 μg/ml	
Cisplatin	18	31	47	85	96	2.76
LH	15	32	44	67	75	3.14
$[CoLCl]\cdot 2H_2O$	12	28	39	52	74	3.69
$[NiLCl\cdot 2H_2O]$	14	31	44	63	72	2.89
[CuLCl]	18	39	58	69	84	1.94
[ZnLCl]	19	34	46	63	76	2.56

5.1. Chemistry

5.1.1. Synthesis of (D-glucopyranose)-4-phenylthiosemicarbazide LH

D-Glucose (0.01 mol) is treated with 4-phenylthiosemicarbazide (0.01 mol) in presence of a small amount of NH_4Cl (0.004 mol) in refluxing methanol. Reaction was monitored through TLC at a time interval of 20 min. The reaction proceeds almost to completion after about 1.5 h. Thus separated product was filtered off washed with methanol and diethyl ether and dried under vacuum. Yield ~65%, Mp 153–156 °C. Elemental analysis: Calculated (found) for $C_{13}H_{19}N_3O_5S$: C, 47.41 (47.39); H, 5.77 (5.72); N, 12.76 (12.73); S, 9.72 (9.69). FT-IR (KBr) cm^{-1}: 3448 (br s) ν(OH), 2978–2897 (m) ν(CH), 1641 (vs) δ(N–H), 1045 (s) ν(C=S). ^1H NMR (DMSO) δ: 10.50 (s, 1H, hydrazide NH), 8.90–9.00 (2s, 1H, glycosylic N–H), 7.12–7.60 (m, 5H, Ar), 4.99 (d, 1H, C–1–H), 4.60 (s, 1H, Ar–C–NH), 3.07–3.67 (m, –OH and –H of saccharide moiety).

5.2. Synthesis of complexes

In a representative preparation, the complex was prepared by the addition of methanolic solution of metal(II) chloride (0.003 mol) with constant stirring to the ligand (0.003 mol) in the same solvent at room temperature. Ammonia gas is passed through the reaction mixture. After a sufficient time of stirring the reaction mixture was concentrated. All the complexes were filtered and washed with methanol and dried in vacuum over P_2O_5.

5.2.1. $[CoLCl]\cdot 2H_2O$

Yield ~70%, Mp > 280 °C. Elemental analysis: Calculated (found) for $[Co(C_{13}H_{18}N_3O_5S)\ Cl].2H_2O$: C, 35.45 (35.41); H, 4.54 (4.50); N, 9.54 (9.49); S, 7.27 (7.24); Co, 13.39 (13.36); Cl, 8.05 (8.01). FT-IR (KBr) cm^{-1}: 3268 (br s) ν(OH), 2920 (m) ν(CH), 1598 (vs) δ(N–H), 1030 (s) ν(C=S). Molar conductance: 31 mho $cm^2 mol^{-1}$. Magnetic moment: 4.5 BM.

5.2.2. $[NiLCl\cdot 2H_2O]$

Yield ~70%, Mp > 280 °C. Elemental analysis: Calculated (found) for $[Ni(C_{13}H_{18}N_3O_5S)\ Cl.2H_2O]$: C, 34.06(33.99); H, 4.80 (4.78); N, 9.17 (9.13); S, 6.98 (6.95); Ni, 12.81 (12.78); Cl, 7.74 (7.71). FT-IR (KBr) cm^{-1}: 3323 (br s) ν(OH), 2932 (m) ν(CH), 1592 (vs) δ(N–H), 1036 (s) ν(C=S). Molar conductance: 29 mho $cm^2 mol^{-1}$. Magnetic moment: 2.96 BM.

5.2.3. [CuLCl]

Yield ~75%, Mp > 280 °C. Elemental analysis: Calculated (found) for $[Cu(C_{13}H_{18}N_3O_5S)Cl]$: C, 36.53(36.59); H, 4.21 (4.19); N,

Table 4
Effect of drugs on body weight changes in tumor induced mice.

Group	Dose (mg/Kg) i.p.	% increase in weight as compared to day-0 (mean ± SE)				
		Day-3	Day-6	Day-9	Day-12	Day-15
Control	–	3.96 ± 1.77	6.84 ± 1.69	16.43 ± 1.45	18.15 ± 1.59	21.25 ± 2.34
Cisplatin	3.5	3.81 ± 1.55	13.04 ± 1.22	18.27 ± 1.22	8.72 ± 2.22[a]	1.53 ± 3.24[a]
LH	50	4.45 ± 1.62	13.29 ± 1.27	19.08 ± 3.41	16.54 ± 2.35	7.43 ± 2.45[a]
[CoLCl]·$2H_2O$	50	3.81 ± 1.21	11.34 ± 1.33	19.46 ± 3.61	18.71 ± 4.33	14.61 ± 1.82
[NiLCl·$2H_2O$]	50	4.21 ± 1.34	12.34 ± 2.56	18.49 ± 3.51	13.56 ± 2.39	7.24 ± 1.65[a]
[CuLCl]	50	4.62 ± 1.48	17.54 ± 2.23	21.32 ± 1.72	16.42 ± 3.03	7.55 ± 3.23[a]
[ZnLCl]	50	4.34 ± 1.45	16.43 ± 1.58	18.32 ± 1.53	16.54 ± 1.65	8.45 ± 2.39[a]

[a] $p < 0.05$ Vs Control.

9.83 (9.79); S, 7.49 (7.45); Cu, 14.88 (14.83); Cl, 8.30 (8.27). FT-IR (KBr) cm^{-1}: 3445 (s) ν(OH), 3335 (s) ν(NH), 2978–2897 (m) ν(CH), 1590 (vs) δ(N–H), 1035 (s) ν(C═S). Molar conductance: 20 mho cm^2mol^{-1}. Magnetic moment: 1.93 BM.

5.2.4. [ZnLCl]

Yield ~75%, Mp > 280 °C. Elemental analysis: Calculated (found) for [Zn($C_{13}H_{18}N_3O_5S$) Cl].$2H_2O$: C, 34.89 (34.82); H, 4.47 (4.45); N, 9.39 (9.33); S, 7.15 (7.12); Ni, 12.81 (12.78); Cl, 7.74 (7.71). FT-IR (KBr) cm^{-1}: 3368 (br s) ν(OH), 2929 (m) ν(CH), 1600 (vs) δ(N–H), 1033 (s) ν(C═S). ^{1}H NMR (DMSO)δ: 10.50 (s, 1H, hydrazide NH), 8.90–9.00 (2s, 1H, glycosylic N–H), 7.12–7.60 (m, 5H, Ar), 4.99 (d, 1H, C–1–H), 4.60 (s, 1H, Ar–C–NH), 3.77–3.07 (m, –OH and –H of saccharide moiety). Molar conductance: 24 mho cm^2mol^{-1}.

5.3. Antitumor activity

5.3.1. Cell lines

Cancer cell lines viz. Ehrlich's Ascitic Carcinoma (EAC), to induce cancer in animal model (mice) were obtained from Amala Cancer Research Center, Amala Nagar, Thrissur, Kerala, India. The cells were maintained as ascites tumor in Swiss albino mice by intraperitoneal inoculation of 1×10^6 viable cells.

5.3.2. Animals

Female Swiss albino mice of 6–8 weeks old (25 ± 5 g body weight) were selected. The animals were acclimatized to the experimental room having temperature 23 ± 2 °C, controlled humidity conditions, and 12:12 h light and dark cycle. The Mice were housed in sterile polypropylene cages containing sterile paddy husk as bedding material with maximum of 4 animals in each cage. The mice were fed on autoclaved standard mice food pellets (Hindustan Lever) and water *ad libitum*. The animal experiments were performed according to the rules and regulations of the Institutional Animal Ethics Committee (IAEC).

5.3.3. Preparation of test solution of compounds

The solutions of the compounds were prepared by suspending them in 2% acacia and administrated intraperitoneally on 3rd, 5th, 7th, 10th, 12th, and 14th day of tumor inoculation in the volume of 0.1 ml/10 g mice. All the compounds were tested at the dose of 50 mg/kg body weight. The dose of cisplatin selected was 3.5 mg/kg. This was calculated by using body mass Index and past experience with the drug [24].

Table 5
Effect of drugs on the survival time in tumor induced mice.

Group	Dose (mg/kg)	LC_{50} in $M \times 10^{-8}$	Mean survival time (days)		
			(Mean ± SEM)	%T/C	%ILS
Control	–	–	19.00 ± 0.37	–	–
Cisplatin	3.5	2.76	38.29 ± 1.17[a]	201.53	101.53
LH	50	3.14	27.83 ± 0.48[a]	146.47	46.47
[CoLCl]·$2H_2O$	50	3.69	22.50 ± 0.43[a]	118.42	18.42
[NiLCl·$2H_2O$]	50	2.89	25.83 ± 0.43[a]	135.95	35.95
[CuLCl]	50	1.94	28.83 ± 0.43[a]	151.74	51.74
[ZnLCl]	50	2.56	27.67 ± 0.42[a]	145.63	45.63

[a] $p < 0.05$ Vs Control Groups.

5.3.4. Determination of cytotoxicity of compounds against EAC cells (In vitro studies, Tryphan Blue Exclusion Method (Cell Viability Test))

In vitro short-term cytotoxic activity of drug was determined using EAC cells. The EAC cells that were collected from the animal peritoneum by aspiration were washed repeatedly with PBS to free it from blood. After checking the viability of the cells in a haemocytometer, a cell (1×10^6) in 0.1 ml PBS, 0.01 ml of various concentrations of test compounds (1–50 μg/ml) were made (the test compounds were dissolved in dimethylsulphoxide (DMSO), the final concentration of DMSO not exceeding 0.1% of the total volume) and phosphate buffered saline (0.1 mole/l, pH 7) in a total volume of 0.9 ml were incubated in clean sterile tubes for 3 h at 37 °C. The control tube had 10 μl of solvent. The final volume was made up to 0.9 ml with PBS. To each 100 μl of Tryphan blue solution was added. The live (without stain) and dead (with blue stain) cells were counted using haemocytometer and percent cell death was calculated using the formula:

$$\%\text{Cytotoxicity} = 100 \times (T_{\text{dead}} - C_{\text{dead}})/T_{\text{tot}}$$

where, T_{dead} is the No. of dead cells in the treated group, C_{dead} is that in the control group and T_{tot} is the total number of dead and live cells in the test compound treated group. Cisplatin was used as the standard [25].

5.3.5. Induction of Ehrlich Ascites Carcinoma [22]

Antitumor activities of compounds were determined using Ehrlich Ascites Carcinoma (EAC) tumor model in mice. Female Swiss albino mice were divided into groups of 6 animals each. (A. Tumor bearing mice, B. Tumor bearing mice treated with one dose of Cisplatin, C. Tumor bearing mice groups treated with compounds). The ascites carcinoma-bearing mice (donor) were used for the study, 15 days after tumor transplantation. The ascitic fluid was drawn using an 18-gauge needle into sterile syringe. A small amount was tested for microbial contamination. Tumor viability was determined by Tryphan blue exclusion test and cells were counted using haemocytometer. The ascitic fluid was suitably diluted in normal saline to get a concentration of 10^6 cells/ml. of tumor cell suspension. This was injected intraperitonealy to obtain ascitic tumor. The mice were weighed on the day of tumor inoculation and then once in three days thereafter. Treatment was started on the 3rd, 5th, 7th, 10th, 12th, and 14th day of tumor inoculation. Cisplatin (one dose) was injected on 1st day intraperitonealy. The animals in each of the groups were kept to check the mean survival time (MST) of the tumor bearing hosts.

Antitumor effects of compounds were assessed by observation of following parameters.

5.3.6. Percentage increase in weight as compare to day-0 weights

The animals are weighed on the day of inoculation and after once in three days in the post inoculation period, the percentage increase in weight was calculated using the formula: % Increase in weight = [(animal weight on respective day/animal weigh on day 0) − 1] × 100 [26].

5.3.7. Mean survival time and increase in life span and statistical analysis

Total number of days an animal survived from the day of tumor inoculation was counted. Subsequently the mean survival time was calculated. The percentage increase in life span was calculated using the formula: ILS (%) = [(Mean survival time of treated group/mean survival time of control group) − 1] × 100 [25]. Results were analyzed by one-way ANOVA by Scheffe's post-hoc test using SPSS computer package.

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