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Synthesis, Spectroscopic Characterization, Antibacterial and Short Term *in vitro* Cytotoxicity Studies of Copper(II) Complexes of Novel Tridentate N,N,S Donor Ligand 2-Benzoylpyridine-N(4),N(4)-(N,N-diethyl-N-methylamine-2,2'-diyl)thiosemicarbazone

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A tridentate N,N,S-donor ligand, 2-benzoylpyridine-N(4),N(4)-(N,N-diethyl-N-methylamine-2,2'-diyl)thiosemicarbazone (Hbptsc) has been synthesized and characterized by elemental CHN analysis, UV-visible, FT-IR and ^{1}H NMR spectroscopy. Copper(II) complexes of the ligand, Hbptsc synthesized have been characterized by elemental analysis, UV-visible spectra, FTIR spectra and EPR spectroscopic simulation. The complexes hold the stoichiometry of the type [CuLX] where X= Cl (1), NO_3 (2), SO_4 (3), N_3 (4), SCN (5) confirmed by the molar conductivity studies of 10^{-3} M solutions in DMF at room temperature. The EPR spectra of the complexes recorded in DMF at 77 K shows an axial type spectra with two distinct g-values, $g_{\parallel}$ and $g_{\perp}$ indicating a four coordinated planar geometry. The antimicrobial studies of the copper(II) complexes shows an appreciable activity against both gram positive and gram negative bacteria using streptomycin as positive control. The short term *in vitro* cytotoxicity studies following trypan blue dye exclusion method exhibits pronounced activity against the Dalton's Lymphoma Ascites tumour cells extruded from the peritoneal cavity of mice.

Keywords: Thiosemicarbazone, 2-Benzoylpyridine, Copper(II) complexes, Antitumour activity.

INTRODUCTION

Thiosemicarbazones and their metal complexes have been extensively studied during recent years mainly because of their various biological properties [1,2]. These complexes show high chelating behaviour especially with the metal ions of the first row transition and main group elements, bonding through sulphur and azomethine nitrogen atoms [3]. Thiosemicarbazones and their transition metal complexes have a wide range of biological activities, some of them being antiviral, antifungal, antibacterial, antitumor anticancerogenic antioxidant besides showing insulin mimetic effects too. In solid state, they exist in the thione form and in solution they exist as an equilibrium mixture of both thione and thienol forms which is essential for explicit chelating behaviour.

Previous studies indicate the planar nature of biologically active thiosemicarbazones with heterocyclic bases giving rise to NNS tridentate system [4]. The third donor atom, present in the ring of heterocyclic thiosemicarbazones (N in the case of the pyridine ring, O in the case of pyridine *N*-oxide) makes them potentially tridentate. The combination of heterocyclic ring with azomethine moiety exerts potential biological and catalytic activities [5]. The activity is found to be highly pronounced where the thiosemicarbazone gets attached through the 2-position of the heterocyclic system and the activity diminishes when the point of attachment is shifted further to 3 or 4-positions presumably due to lower coordination ability. In this work we report the synthesis, spectral characterization and short term *in vitro* cytotoxicity results of the ligand, Hbptsc and its copper(II) complexes.

EXPERIMENTAL

2-Benzoylpyridine (Aldrich), N-Methyl piperazine (Aldrich), $CuCl_2{\cdot}2H_2O$, $CuSO_4{\cdot}5H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Cu(OAc)_2{\cdot}H_2O$, NaN_3 and KSCN (E. Merck) were used as received. All the solvents were distilled before use.

2-Benzoylpyridine-N(4),N(4)-(N,N-diethyl-N-methyl-amine-2,2'-diyl)thiosemicarbazone (Hbptsc) was synthesized by the adoption of a reported procedure [6]. Elemental CHN analyses (C, H and N) were carried out using Elementar Vario EL III analyzer. Infrared spectra were recorded on a Thermo Nicolet Avatar FTIR spectrometer as KBr pellets in the range 4000-400 cm^{-1}. Solid state electronic spectra were recorded in the 900-250 nm on a Varian Carry 5000 UV-VIS-NIR spectrophotometer. ^1H NMR spectra were obtained in a Bruker 400 Avance III FT NMR spectrometer instrument using $CDCl_3$-d_6 as the solvent and TMS as the internal reference. The EPR spectra (X-Band) were recorded at liquid nitrogen temperature (LNT) (77 K) in DMF using ESR-JEOL Japan instrument. Magnetic susceptibility studies were carried out using vibrating sample magnetometer at room temperature. PXRD diffraction studies were carried out using Bruker AXS D8 Advance diffractometer and SEM studies by using Jeol 6390LV.

Synthesis of ligand, 2-benzoylpyridineN(4),N(4)-(N,N-diethyl-N-methylamine-2,2'-diyl)thiosemicarbazone (Hbptsc): A solution of N(4)-methyl, N(4)-phenyl thiosemicarbazide (1 g, 5.52 mmol) dissolved in 10 mL hot methanol was treated with N-methyl piperazine (0.62 mL, 5.52 mmol) and 2-benzoyl pyridine (1.011 g, 5.52 mmol) dissolved in 5 mL methanol and the resulting solution was heated under reflux for 1 h at about 50 °C. The solution was chilled and the microcrystals were separated, washed with methanol and dried over P_4O_{10} in vacuum. The deep yellow microcrystals of Hbptsc were obtained in 50 % yield were recrystallized from methanol, m.p. 178 °C. Anal. calcd. (Found) %: C, 63.71 (63.52); H, 6.19 (6.47); N, 20.64 (20.58) UV-visible, cm^{-1}: 36630 s, 25706; IR (cm^{-1}): 1575 s, 996 m, 1397 s, 791 sh. ^1H NMR ($CDCl_3$–TMS) (ppm): N(3)H, 13.713s; C(1)H, 8.797d; C(2)H, 7.57d; C(3)H, 7.812d; C(4)H, 7.797d; C(8)-C(12), 7.21-7.70m; C(14) & C(16), 2.585, d; C(15) & C(17), 2.575, d; C(18)3H, 4.316 s.

Preparation of Cu(II) complexes: All the Cu(II) complexes were synthesized by refluxing equimolar amounts of the ligand, Hbptsc and the hydrated metal salts in methanol for 3-5 h. The complexes were filtered, washed with ethanol and finally dried in vacuum over P_4O_{10}.

Synthesis of [Cu(bptsc)Cl] (1): Methanolic solutions of the ligand, Hbptsc (1 mmol, 0.34 gm) and $CuCl_2{\cdot}2H_2O$ (1 mmol, 0.17 g) were mixed together and heated under reflux for about 3 h. The resulting solution was allowed to stand at room temperature for slow evaporation. Dark green coloured micro crystals formed were separated, washed with methanol and dried over P_4O_{10} in vacuum elemental analyses for $C_{18}H_{20}N_5SCuCl$: Calcd. (%): C, 49.42; H, 4.57; and N, 16.01. Found (%): C, 49.21; H, 4.51; and N, 15.59.

Synthesis of [Cu(bptsc)(NO_3)] (2): The ligand Hbptsc (1 mmol, 0.34 g) dissolved in 20 mL hot methanol was refluxed with methanolic solution $Cu(NO_3)_2{\cdot}3H_2O$ (1 mmol, 0.24 g) for 2 h. The resulting solution was allowed to stand at room temperature for slow evaporation. Dark green coloured microcrystals of the complex **2** were filtered, washed with methanol and dried over P_4O_{10} in vacuum. Elemental analyses for $C_{18}H_{20}N_6SO_3Cu$: Calcd. (%): C, 46.60; H, 4.31; and N, 18.12. Found (%): C, 46.68; H, 4.57; and N, 18.23.

Synthesis of $[Cu_2(bptsc)_2(SO_4)].2H_2O$ (3): $CuSO_4{\cdot}5H_2O$ (1 mmol, 0.25 g) dissolved in methanol-water mixture was added to Hbptsc (1 mmol, 0.34 g) dissolved in 20 mL hot methanol and the mixture was refluxed for 6 h. The resulting solution was allowed to stand at room temperature for slow evaporation, which yielded dark blue coloured microcrystals of the complex **3**. It was filtered washed with methanol and dried over P_4O_{10} in vacuum. Elemental analyses for $C_{36}H_{44}N_{10}S_3O_6Cu_2$: Calcd (%): C, 47.88; H, 4.87; and N, 15.50. Found (%): C, 47.60; H, 4.54; and N, 15.76.

Synthesis of [Cu(bptsc)N_3] (4): $Cu(CH_3COO)_2{\cdot}H_2O$ (1 mmol, 0.2 g) dissolved in 20 mL methanol was refluxed with Hbptsc (1 mmol, 0.34 g) in methanol and to the refluxing solution sodium azide, NaN_3 (1 mmol, 0.0650 g) dissolved in methanol was added and further refluxed for 3 h. The resulting solution was allowed to stand at room temperature and on slow evaporation gave dark green coloured crystals of the complex **4** which are filtered, washed with methanol and dried over P_4O_{10} in vacuum. Elemental analyses for $C_{18}H_{20}N_8SCu$: Calcd. (%): C, 48.70; H, 4.51; and N, 25.25. Found (%): C, 48.45; H, 4.51; and N, 25.19.

Synthesis of [Cu(bptsc)(NCS)] (5): $Cu(CH_3COO)_2{\cdot}H_2O$ (1 mmol, 0.2 g) dissolved in 20 mL methanol was refluxed with Hbptsc (1 mmol, 0.34 g) in methanol and to the refluxing solution KSCN (1 mmol, 0.097 g) dissolved in methanol was added and further refluxed for 4 h. On slow evaporation dark green coloured crystals of complex **5** separated out, filtered, washed with methanol and dried over P_4O_{10} in vacuum. Elemental analyses for $C_{19}H_{20}N_6S_2Cu$: Calcd. (%): C, 49.61; H, (4.35; and N, 18.28. Found (%): C, 49.15; H, 4.49; and N, 18.71.

RESULTS AND DISCUSSION

All the complexes are dark blue or green in colour and soluble in solvents like methanol, ethanol, chloroform and DMF. The molar conductivities of 10^{-3} M DMF solutions of the complexes indicate they are non-conductors with values less than 20 Ω^{-1} mol^{-1} cm^{-1}. The partial elemental analysis data and conductance measurements are consistent with the general formulation [CuLX] where X= Cl (1), NO_3 (2), SO_4 (3), N_3 (4), SCN (5). For the compound **3**, the elemental analysis data matches with the stoichiometry containing two molecules of water of crystallization/lattice water. Magnetic susceptibility measurements of the copper(II) complexes at room temperature lie between 1.80-1.99 BM indicating a mononuclear copper centre in all the complexes, characteristic of one unpaired electron for a d^9 configuration [18].

^1H NMR spectrum of ligand (Hbptsc): ^1H NMR spectrum of the ligand Hbptsc recorded in $CDCl_3$ is given in Fig. 1. A sharp singlet at a downfield value of δ 13.71 is due to the hydrogen atom bonded to the third nitrogen atom, N(3)H. The ^1H NMR spectrum reveals four signals for the pyridyl moiety, multiplet for the phenyl moiety and two well-resolved peaks for the piperazine moiety [7]. The phenyl moiety appears as a multiplet at about 7.70-7.21 ppm where the chemical shift values are very close and hence it is very difficult to be resolved. A singlet at 4.316 ppm is assigned to the C(18)H, of the CH_3 group attached to the electronegative nitrogen atom in the

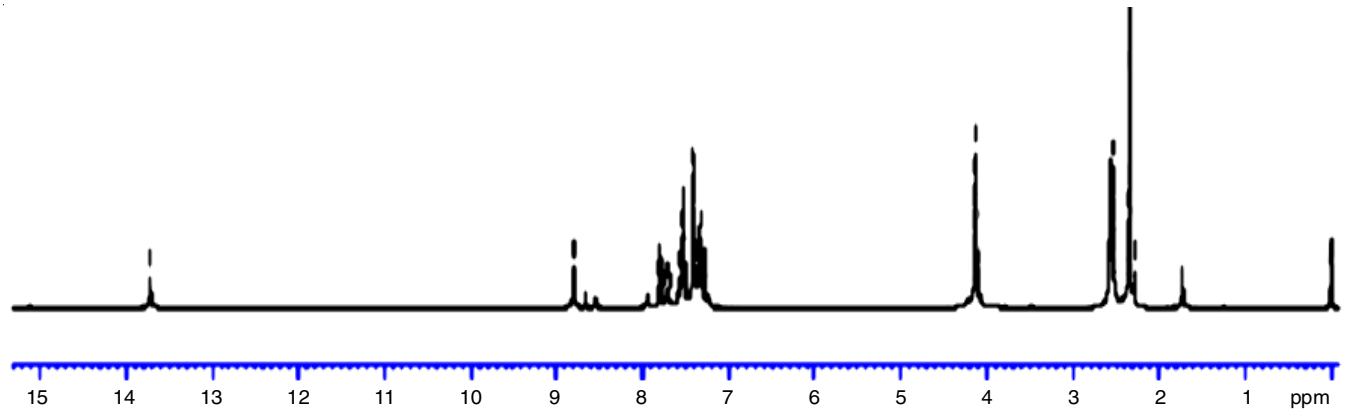


Fig. 1. ^{1}H NMR spectrum of Hbptsc in $CDCl_3$

piperazine moiety. A doublet at 8.797 ppm of pyridyl proton N(1)H has been identified. The equatorial C(14)H and C(16)H Protons resonates at 2.585 ppm while the axial C(15)H and C(17)H protons at 2.575 ppm, respectively in the piperazine moiety.

IR spectra: Table-1 shows the tentative IR assignments of ligand Hbptsc and its copper(II) complexes. The ligand Hbptsc exhibits a broad medium band at 3050 cm^{-1} attributed to ν(NH) vibration of thiosemicarbazone moiety [8]. The absence of this band in the spectra of complexes indicates the ligand coordination around the metal ion in its deprotonated thiolate form. A medium band at 1575 cm^{-1} corresponding to ν($^{6}C=^{2}N$), shifts to higher wave numbers in the complexes due to the overlapping of ν($^{6}C=^{2}N$) with newly formed $^{3}N=^{13}C$ bond as a result of coordination [8]. A medium band at 1397cm^{-1} corresponding to ν($^{13}C=^{1}S$) in the free ligand shifts to lower wave numbers in complexes, indicating the decrease in the $^{13}C=^{1}S$ bond order due to chelation and delocalization effects [9]. The shift of the pyridine ring in-plane and out of plane bending vibrations at 791 and 640 cm^{-1} in the free ligand shows a positive shift in complexes suggesting the coordination of metal to ligand *via* pyridine nitrogen [10-13].

The nitrato complex **2** shows bands at 1420, 1300 and 1030 cm^{-1} corresponding to the ν_5, ν_1 and ν_2, respectively, with a separation of 120 cm^{-1} between the highest two vibrations indicating the coordination to the metal *via* the terminally bonded monodentate nitrato groups [14].

The sulfato complex **3** with four fundamental vibration modes appear as bands of medium intensity. ν_1 band at 970 cm^{-1} appears with medium intensity while three weak bands of ν_3 at 1163, 1096 and 1053 cm^{-1} further indicate a bridging bidentate mode of coordination to copper(II) ion [14].

For azido complex **4**, a strong band at 2040 cm^{-1} may be assigned to asymmetric stretching vibration of N(3) and a medium band at 1383 cm^{-1} assigned to (N(3) symmetric stretching mode. A medium band at 650 cm^{-1} corresponds to δ (N-N-N) vibrations suggesting that Cu-N-N-N bond is not linear [14].

The thiocyanato complex **5** shows a strong band below 2050 cm^{-1} at 2042 cm^{-1} characteristic of the ν(CN) vibrations due to coordination through the nitrogen atom. A weak band at 787 cm^{-1} corresponds to the ν(CS) vibrations for the N-bonded complex in agreement with the available reports. A sharp band appears at 480 cm^{-1} corresponding to δ (NCS) indicating Cu-N bonding rather than Cu-S bonding in the complex [14].

Electronic spectra: Table-2 shows the electronic spectral assignments of ligand, Hbptsc and its copper(II) complexes. The ligand exhibits a broad band around 36,000 cm^{-1} assigned to the $\pi' \rightarrow \pi^*$ transition of pyridyl ring and imine function of the thiosemicarbazone moiety. The shift of the $\pi' \rightarrow \pi^*$ bands to the longer wavelength in complexes is a result of decrease of C=S bond order due to conjugation following complexation [15]. The peak at about 25,000 cm^{-1} for the free ligand is attributed to $n \rightarrow \pi^*$ transition corresponding to the transition of the thioamide function. The $n \rightarrow \pi^*$ transitions shift to higher energy on complexation due to the coordination *via* pyridyl nitrogen. Weak bands observed at about 23000 and 26000 cm^{-1} are assigned to N(pyridyl)→ Cu(II) and S→Cu (II) charge transfer transitions, respectively [16]. Metal-ligand charge transfer bands for the complexes appears as a combination of both S→Cu(II) and N(pyridyl)→ Cu(II) transitions. The bands which appear as weak shoulders centred around 16,000 cm^{-1} are assigned to *d-d* transitions suggestive of complexes with a square planar geometry. The square planar type copper(II) complexes with $d_{x^2-y^2}$ ground state usually exhibit three transitions *viz.* d_{xz}, $d_{yz} \rightarrow d_{x^2-y^2}$, $d_{xy} \rightarrow d_{x^2-y^2}$, $d_{z^2} \rightarrow d_{x^2-y^2}$. These are difficult to be resolved since all the three possess more or less same energies [17-19].

TABLE-1
KEY IR SPECTROSCOPIC DATA (cm^{-1}) OF Hbptsc AND ITS COPPER(II) COMPLEXES

Compound	ν(N–H)	ν(C=N) + ν(N=C)	ν(N–N)	ν(C=S)	δ(C=S)	δ(o.p)
Hbptsc	3050s	1575s	996w	1397s	791w	640w
[Cu(bptsc)Cl]	–	1598w	1015w	1376s	885m	703w
[Cu(bptsc)(NO_3)]	–	1589w	1030w	1377s	884w	647m
[Cu_2(bptsc)$_2$(SO_4)]·2H_2O	–	1595w	1031sh	1315s	882w	699w
[Cu(bptsc)N_3	–	1592m	1034 m	1383m	881m	690w
[Cu(bptsc)(NCS)]	–	1588w	1040w	1371s	882w	696w

TABLE-2
ELECTRONIC SPECTRAL ASSIGNMENTS (cm^{-1}) OF Hbptsc AND ITS COPPER(II) COMPLEXES

Compound	Mode	*d-d*	CT	N→π*	π→π*
Hbptsc	Solid	–	–	25706 s	36630 s
[Cu(bptsc)Cl]	Solid	16393(sh)	23980 (sh)	29069 s	33444sh
[Cu(bptsc)(NO_3)]	Solid	16501 (sh)	22978 s	29411(sh)	35460 s
[Cu_2(bptsc)$_2$(SO_4)]·2H_2O	Solid	16051 (sh)	26455, 2267599 (sh)	29673 (sh)	34843 s
[Cu(bptsc)N_3	Solid	16077 (sh)	26246,22988 s	28409 s	35971 s
[Cu(bptsc)(NCS)]	Solid	17152,16583(sh)	23474 s	27624 (sh)	35714 sh

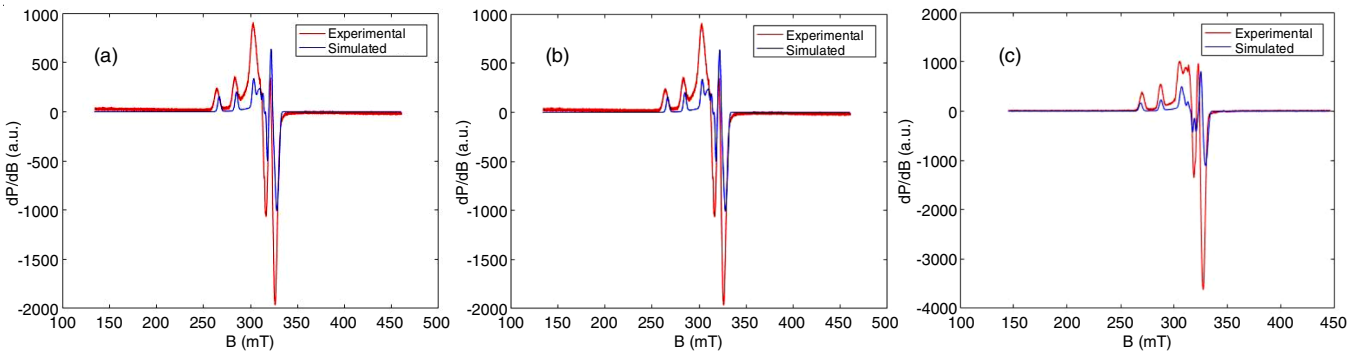


Fig. 2. EPR spectra of (a) [Cu(bptsc)NO_3]; (b) [Cu(bptsc)N_3]; (c) [Cu(bptsc)NCS] at 77 K

EPR spectra: The simulated X-band EPR spectra of three copper(II) complexes recorded at 77 K liquid nitrogen temperature (LNT) in DMF are shown in Fig. 2a-c. The g-value, the Lande splitting factor of an electron in complexes undergoes a shift from the typical g-value of a free electron, *viz*., 2.0023. This forms strong evidence about the geometry and the extent of metal exchange interactions in the complex between the metal and the ligand. The EPR spectra of all the complexes shows four hyperfine lines due to the interaction of electron spin with nuclear spin (^{65}Cu, I = 3/2) indicating an axial type spectra. The four well resolved hyperfine lines are suggestive of monomeric state of complexes with one copper centres. The hyperfine splitting due to azomethine nitrogen and nitrogen atom of the coordinated heterocyclic base geometry. For all the complexes, the geometric parameter value G, falls in the range between 3.5 and 4 and $g_{\parallel} > g_{\perp}$ value points towards a $d_{x^2-y^2}$ ground state. The $g_{\parallel}$, $g_{\perp}$, g_{av}, $A_{\parallel}$, $A_{\perp}$ and the energies of *d-d* transitions have been used to calculate the bonding parameters β^2, β^2, γ^2, which may be regarded as measures of covalency of the in-plane σ bonds, in-plane π bonds and out of plane π bonds, respectively. $g_{\parallel} < 2.3$ indicates significant covalent bonding in these complexes and similar $g_{\parallel}$ values give an idea that the bonding is dominated by thiosemicarbazone moiety rather than the heterocyclic bases. The value of in-plane σ-bonding parameters β^2 was estimated from the expression [20,21].

$$\beta^2 = -A_{\parallel}/0.036 + (g_{\parallel} - 2.0023) + 3/7\ (g_{\perp} - 2.0023) + 0.04$$

Hathaway and Billing [19] has shown that $K_{\parallel} \approx K_{\wedge} = 0.77$, for pure σ bonding and for in plane π bonding, $K_{\parallel} < K_{\perp}$ and for out of plane π bonding, $K_{\parallel} > K_{\perp}$. Also values of bonding parameters falling below 1 are the indication of the diminishing ionic character with increased covalent character. Hence the evaluated values for β^2, β^2, γ^2 of the complexes are consistent with both strong in plane σ and in plane π bonding.

Scanning electron microscopic (SEM) studies: The scanning electron microscopic image of the ligand, Hbptsc is shown in Fig. 3 and this shows a regular grain like microcrystalline structure.

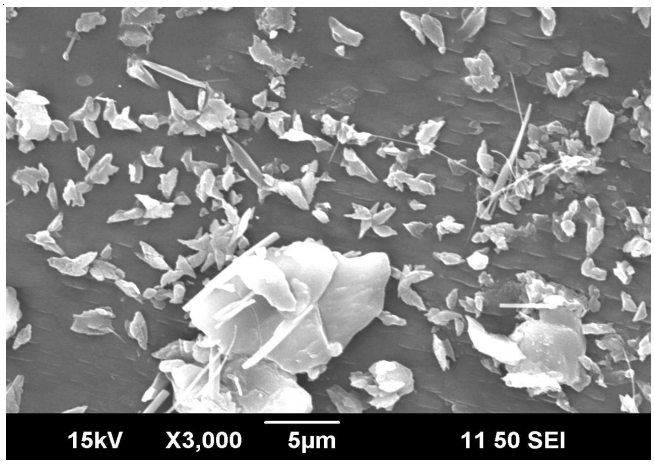


Fig. 3. SEM image of ligand

PXRD data: Fig. 4a-b shows the PXRD patterns of ligand, Hbptsc and Cu(bptsc)NO_3 complex with well defined peaks. indicating their crystalline nature. The diffractogram of ligand recorded 12 reflections, 2θ ranging from 8° to 23° with maxima at 2θ = 23° which corresponds to interplanar spacing d = 0.3825 nm. Twelve reflections were recorded for the metal complex, 2θ ranging from 7° to 28° with maxima at 2θ = 28° which corresponds to interplanar spacing d = 0.3138 nm. The particle size of ligand and metal complexes has been calculated using Scherrer's formula $d_{XRD} = 0.9\lambda/(\beta\cos\theta)$, where λ is the wavelength, β is the full width at half maxima and θ is the diffraction angle. The particle sizes have been found to be 29 and 30 nm for the ligand and the metal complexes, respectively [22,23].

The following tentative structures may be proposed for the complexes.

***in vitro* Antibacterial studies:** The inhibition zones produced by the copper(II) complexes of the ligand Hbptsc against

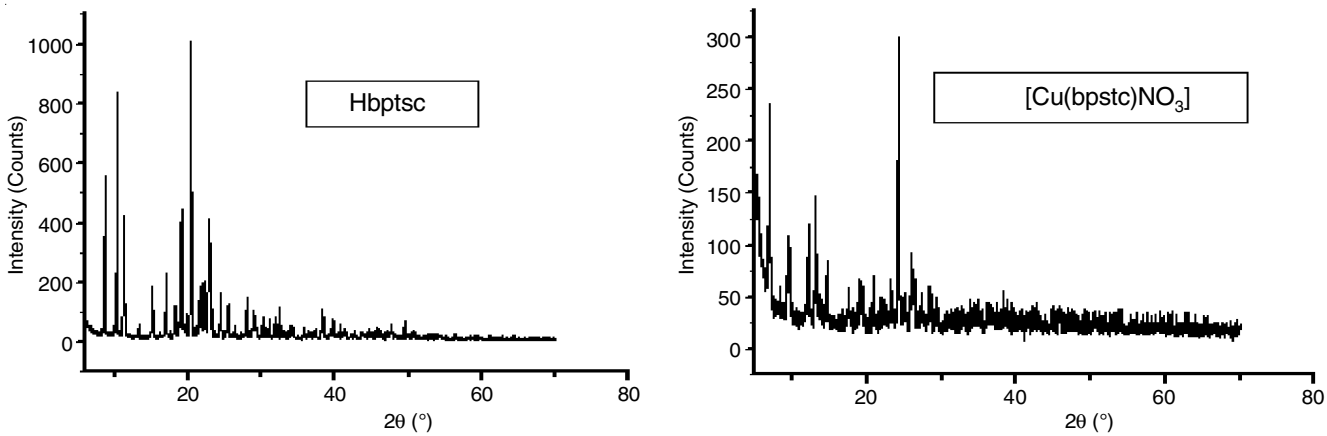
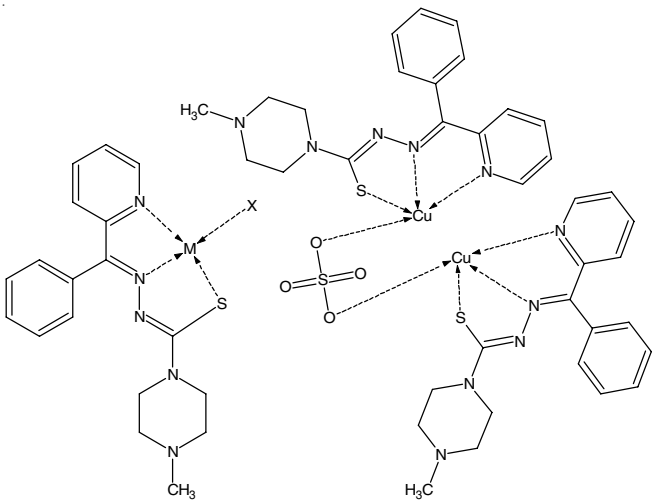


Fig. 4. Powder X-ray diffractogram of ligand and $[Cu(bptsc)NO_3]$



the bacterial strains is shown in Table-3. These complexes have been tested against *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans* (MTCC 890) following the agar well diffusion method, using streptomycin as the positive control. The respective activities are evaluated by measuring the diameters of the inhibition zone exhibited by the tested compounds at two concentrations *viz.* 250 and 1000 µg/mL in DMSO.

Out of four strains chosen at two different concentrations, $[Cu(bptsc)NO_3]$ is found to be active against all strains of bacteria exhibiting the largest inhibition zone of 3.0 and 3.5 cm, respectively at 250 and 1000 µg/mL for *E. coli*.

A possible mode of toxicity could be speculated in the light of chelation theory [24,25]. The chelation reduces the polarity of the metal ion, mainly because of the partial sharing of its positive charge with the donor group and a possible π electron delocalization over the whole chelate ring [25]. Besides chelation, higher toxicity of the metal complexes could be attributed to the involvement of metal ion in the normal cell process [26]. The cell walls and membranes made up of lipids and polysaccharides are the most preferred centres for metal ion interaction. A decrease in polarity further increases the lipophilic character of the chelate favouring the interaction between the metal ion and the lipid. This leads to the breakdown of the permeability barrier of the cell resulting in interference with the normal cell processes. The geometry and charge distribution around the molecule should be compatible with the pores of the bacterial cell wall, so as to facilitate the penetration of the toxic agent through the cell wall. The interaction of metal ion with cellular compounds are due to the fact that all these structures contain a variety of functioning groups that can act as metal binding agents [26]. The presence of polar substituent's and anion coordination also adds to the antibacterial activity [26-29].

Short term *in vitro* cytotoxicity studies: The short term *in vitro* cytotoxicity results of copper(II) complexes of Hbptsc against Daltons Lymphoma ascites cells (DLA) is shown in Table-4. The studies have been carried out following the trypan blue exclusion method in DMSO at different concentrations of test samples based on the principle that living cells remain unstained due to the prevention of the entry of the dye, trypan blue into the cell through the cell membrane.

TABLE-3
MINIMUM INHIBITORY CONCENTRATIONS (cm's) OF THE COPPER(II) COMPLEXES OF Hbptsc USING THE AGAR-WELL DIFFUSION METHOD

Bacterial culture	[Cu(bptsc)Cl]		[Cu(bptsc)(NO3)]		[Cu2(bptsc)2(SO4)]·2H2O		[Cu(bptsc)N3]		[Cu(bptsc)(NCS)]	
	25 µg/mL	100 µg/mL	25 µg/mL	100 µg/mL	25 µg/mL	100 µg/mL	25 µg/mL	100 µg/mL	25 µg/mL	100 µg/mL
Gram-positive										
S. aureus	1.4	2.0	1.3	2.0	–	–	–	1.4	3.1	3.6
S. mutans	1.8	2.5	2.8	3.2	2.3	3.1	1.5	1.9	1.5	2.4
Gram-negative										
P. aeruginosa	–	1.5	1.0	1.6	–	1.3	1.4	1.0	1.7	–
E. coli	3.0	3.4	3.0	3.5	2.8	3.3	–	1.2	2.0	–

TABLE-4
SHORT TERM *in vitro* CYTOTOXICITY RESULTS OF THE COPPER(II) COMPLEXES OF Hptsc

Concentration (μg/mL)	Cytotoxicity of complexes (%)				
	[Cu(bptsc)Cl]	[Cu(bptsc)NO_3]	[Cu(bptsc)SO_4]·$2H_2O$	[Cu(bptsc)N_3]	[Cu(bptsc)NCS]
10	52	37	36	48	56
20	58	40	42	65	62
50	64	48	50	70	70
100	75	52	62	76	86
200	90	65	72	88	92

The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed repeatedly with phosphate buffered saline (PBS) solution to free it from blood. The viability of the cells was checked in a haemocytometer. The viable cells suspension (1×10^6 tumour cells/0.1 mL PBS) were incubated in clean sterile tubes with the test compounds (25, 50, 100, 200 μg/mL in dimethyl sulfoxide) for 3 h at 37 °C, keeping the final volume as 1.0 mL. The control tube contained only cell suspension. The cell suspension was mixed with 0.1 mL of 1 % trypan blue and kept for 2-3 min and loaded on a haemocytometer. The live (without stain) and dead (with blue stain) cells were counted using haemocytometer and percent cell death was calculated:

$$\text{Cytotoxicity (\%)} = \frac{\text{No. of dead cells}}{\text{No. of live cell + No. of dead cell}} \times 100$$

The results show a very interesting trend of the cytotoxicity by the complexes in the following order: [Cu(bpstc)NCS] = [Cu(bptsc)N_3] > [Cu(bptsc)SO_4 > [Cu(bptsc)Cl] > [Cu(bptsc)NO_3].

Conclusion

A novel heterocyclic Schiff base ligand, Hbptsc and its copper(II) complexes have been synthesized and characterized on the basis of various physico-chemical and spectral studies. The ligand behaves as a neutral tridentate chelating agent coordinating through the azomethine nitrogen, nitrogen atom of the pyridine ring and sulphur atom in the thiosemicarbazone moiety. The spectral and EPR studies invariably reveal a four coordinated planar geometry for the complexes. The XRD patterns of the free ligand and the copper(II) complex have been recorded and the particle sizes are in the nanocrystalline scale. Antibacterial and short term *in vitro* cytotoxicity studies further confirm a planar geometry for all the copper(II) complexes exhibiting pronounced cytotoxicity against the tumour cells of mice.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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