A Comparative Study of The Antibacterial And Short Term In Vitro Cytotoxicity Studies of The Copper(II) Complexes of A Tridentate N,N,S Donor Ligand(HBPSTC) Derived From 2-Benzoylpyridine And Piperazine Base

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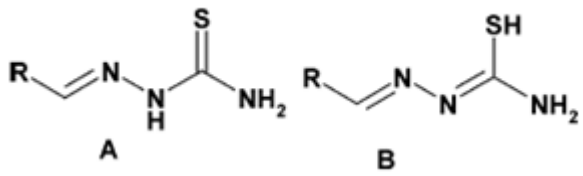
Abstract- *Five copper(II) complexes of the N, N, S-donor ligand* ***Hbpstc*** *were synthesised by refluxing equimolar amounts of the ligand and the copper(II) metal salts in methanol. The ligand ,****Hbptsc*** *was prepared by the reported procedure of Scovill et al. Both the ligand as well as the complexes were characterized by elemental CHN analysis. Molar conductivity studies of the complexes in 10^{-3}M solutions in DMF at room temperature showed that the complexes were non electrolytes with the stoichiometry of the type [CuLX] where X= Cl(1), NO_3(2), SO_4 (3), N_3(4), SCN(5) confirmed by very low molar conductance values. The complexes were tested for their antimicrobial activities using streptomycin as positive control against gram positive and Gramnegative bacteria. The short term in vitro cytotoxiivty studies. Studies of the copper (II) complexes were done following trypan blue dye exclusion method against the Dalton's Lymphoma Ascites tumour cells extruded from the peritoneal cavity of mice.*

Keywords- Thiosemicarbazone, 2-Benzoylpyridine, Copper (II) complexes, EPR,,antimicrobial.

I. INTRODUCTION

Thiosemicarbazones and their metal complexes have been extensively studied during recent years mainly because of their various biological properties [1,2]. These show very 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 [4], antibacterial [5,6], antitumor [7,8], anticancerogenic [9,10], antioxidant [11] besides showing insulin mimetic effects too [12]. In solution these exist as an equilibrium mixture of both thione (A) and thienol forms(B) essential for explicit chelating behaviour, while in solid state, exist in the thione form.



The well documented biological activities of several heterocyclic thiosemicarbazones have been often attributed to a chelation phenomenon with transition metal ions [13]. Past studies indicate the planar nature of biologically active thiosemicarbazones with heterocyclic bases giving rise to NNS tridentate system [14] . Heterocyclic thiosemicarbazones with a functional group attached at the 2-position have been the subject of extensive investigation. Changing the attachment of the thiosemicarbazone moiety to the 3-position on the heteroaromatic ring often causes a decrease in activity, presumably due to a lesser ability for coordination [15], The combination of heterocyclic ring with azomethine moiety exerts potential biological and catalytic activities [15-17].

Copper exhibits considerable biochemical action either as an essential trace metal or as a constituent of various exogenously administered compounds in humans. In its former role it is bound to ceruloplasmin, albumin, and other proteins, while in its latter it is bound to ligands of various types forming complexes that interact with biomolecules, mainly proteins and nucleic acids. The multifaceted role of copper in biological systems is demonstrated by several studies.[18] In this work we report the antibacterial and short term in vitro cytotoxicity studies of the copper (II)

complexes of a novel thiosemicarbazone based tridentate ligand synthesised from 2-Benzoylpyridine and piperazine base.

II. EXPERIMENTAL

2.1. Materials and Measurements

2Benzoylpyridine(Aldrich),NMethylpipierazine,$CuCl_2.2H_2O$,$CuSO_4.5H_2O$,$Cu(NO3)_2.3H_2O$,$Cu(OAc)_2.H2O$, NaN_3, and KSCN (E.Merck) were used as received. All the solvents were distilled before use. Elemental CHN analyses (C, H and N) of the copper(II) complexes were carried out using Elementar Vario EL III analyzer.

2.2 Synthesis of ligand, 2-Benzoylpyridine–N(4),N(4)-(N,N-diethyl N-Methylamine-2,2'-diyl) thiosemicarbazone *(Hbptsc)*

The ligand, ***Hbptsc*** was prepared by the already reported method of J.Scovill et al [19]. 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-methylpiperazine(0.62ml,5.52mmol) and 2-BenzoylPyridine (1.011g,5.52mmol) dissolved in 5 ml methanol and the resulting solution was heated under reflux for 45 minutes at ~50°C. The solution was chilled and kept overnight. Deep yellow microcrystals of *Hbptsc* separated out were filtered, washed well and, recrystallized from methanol, filtered and dried over P_4O_{10} *in vacuo.*

Elemental analyses for $C_{18}H_{21}N_5S$ Anal. Calcd (Found) %: C, 63.71(63.52); H, 6.19 (6.47); N, 20.64 (20.58)

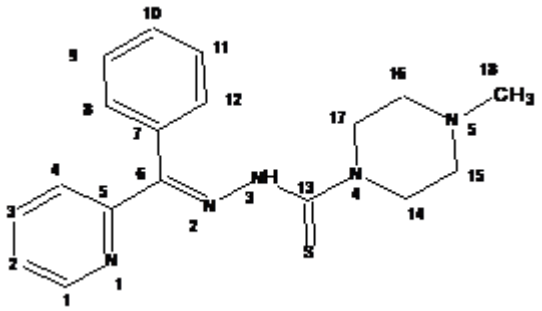


Fig.1, Structure of the ligand, Hbptsc

2.3 Synthesis of complexes

All the complexes were synthesised by refluxing equimolar amounts of the ligand, ***Hbptsc*** and the metal salts of copper (II) ion in methanol for 3-5 hours.

2.3.1. Synthesis of Cu(bptsc)Cl ***(1)***

Methanolic solutions of the ligand ***Hbptsc*** (1mmol, 0.34 gm) and $CuCl_2.2H_2O$ (1 mmol, 0.17g) were mixed together and heated under reflux for about 3 hours. The resulting solution was allowed to stand at room temperature for slow evaporation. Dark green coloured micro crystals of ***1*** separated out were washed, with methanol and dried over over P_4O_{10} *in vacuo.* 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.

2.3.2.. Synthesis of [Cubptsc(NO_3)] ***(2)***

The ligand ***Hbptsc*** (1mmol, 0.34 g) dissolved in 20 ml hot methanol was refluxed with methanolic solution $Cu(NO_3)_{2..}3H_2O$(1mmol, 0.24g) for 2 hours. The resulting solution was allowed to stand at room temperature for slow evaporation. Dark green coloured microcrystals of ***2*** separated out were filtered, washed with methanol and dried over P_4O_{10} *in vacuo*. 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.

2.3.3 Synthesis of [Cu_2(bptsc)$_2$(SO_4).2H_2O] ***(3).***

$CuSO_4.5H_2O$ (1 mmol, 0.25 g) dissolved in methanol-water mixture was added to ***Hbptsc*** (1mmol, 0.34 g) dissolved in 20ml hot methanol and refluxed for 6 hours. The resulting solution was allowed to stand at room temperature for slow evaporation which yielded dark blue coloured microcrystals of ***3.*** It was filtered washed with methanol and dried over P_4O10 *in vacuo.* Elemental analyses for $C_{36}H_{44}N_{10}S_3O_6Cu_2$: Calcd (%): C, 46.11; H, 4.70; and N, 14.97. Found (%): C, 46.39; H, 4.54; and N, 15.26.

2.3.4. Synthesis of [Cu(bptsc)N_3] ***(4).***

$Cu(OAC)_2.H_2O$ (1mmol, 0.2 g) dissolved in 20ml methanol was refluxed with **H*bptsc*** (1mmol, 0.34g) in methanol and to the refluxing solution sodium azide ,NaN_3 (1mmol, 0.0650g) dissolved in methanol was added and further refluxed for 3 hours. The resulting solution was allowed to stand at room temperature and on slow evaporation dark green coloured crystals of ***4*** separated out, filtered, washed with methanol, and dried over P_4O_{10} *in vacuo.*

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.

2.3.5. Synthesis of [Cu(bptsc)(NCS)] ***(5)***

$Cu(OAc)_2.H_2O$ (1mmol, 0.2 g) dissolved in 20ml methanol was refluxed with ***Hbptsc*** (1mmol, 0.34g) in methanol and to the refluxing solution KSCN (1mmol, 0.097g) dissolved in methanol was added and further refluxed for 4 hours. On slow evaporation dark green coloured crystals of ***5*** separated out, filtered, washed with methanol, and dried over P_4O_{10} *in vacuo.* 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.

2.4 Biological Studies

2.4.1 Antibacterial assay

The antibacterial potency of the copper (II) complexes of 2-Benzoylpyridine- N(4),N(4)-(N,N-diethyl-N-methylamine-2,2'-diyl) thiosemicarbazone, ***Hbptsc*** were tested following the Agar Well diffusion method, using streptomycin as the positive control. The respective activities were evaluated by measuring the diameters of the inhibition zone exhibited by the tested compounds at two concentrations viz. 25µg/ml and 100µg/ml in DMSO. Two Gram positive organisms namely Staphylococcus *aureus* (ATCC 25923), Streptococcus *mutans* (MTCC 890) and two Gram negative organisms Pseudomonas *aeruginos*a (ATCC 27853), and Escheria.*coli* (ATCC 25922), were chosen for the study.

2.4.2. In vitro Cytotoxicity studies

The short term *in vitro* cytotoxicity studies of the copper (II) complexes of ***Hbptsc*** against DLA, Daltons Lymphoma ascites cells (DLA) were carried out following the trypanblue exclusion method in DMSO at different concentrations of test samples. This is based on the principle that living cells remain unstained due to the prevention of the entry of the dye, trypan blue into the cellthrough cell membrane. Hence they can be distinguished from the dead cells which get stained due to the entry of the dye into the cell through the disrupted membrane.

2.5 Scanning Electron Microscopic (SEM) studies

The ligand, ***Hbptsc*** and the complex $[Cu(bptsc)NO_3]$ were subjected to SEM studies at different magnifications.and are shown in Fig.2(a) and 2(b) respectively..

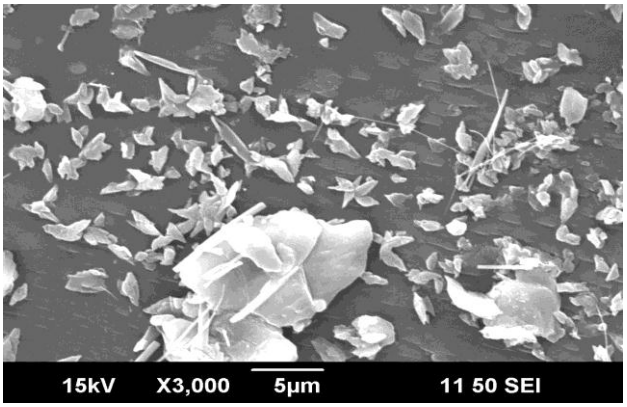


Fig.2(a) SEM photograph of ligand, Hbptsc

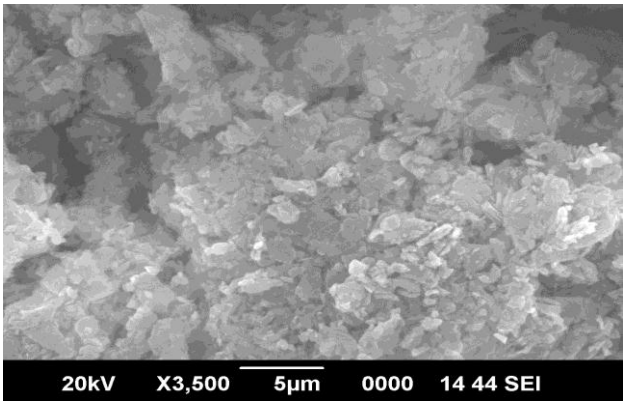


Fig.2(b)SEM photograph of $Cu(bptsc)NO_3$

III. RESULTS AND DISCUSSION

The ligand ***Hbptsc*** interacts with copper (II) ions in the molar ratio 1:1 for all the complexes from ***1-5*** in the presence of anions exhibiting a stoichiometry of the type [Cu(bptsc)X].. All the complexes are partially soluble in non-polar solvents and completely soluble in DMF and DMSO. The complexes separated out from the solution as dark blue or green microcrystals. Conductivity measurements in 10^{-3} M solution of DMF indicate a non-electrolytic nature for the complexes evident from teh molar conductance values falling below 20Ω-1cm-1. Elemental analysis data are consistent with 1:1:1 molar ratio of ***metal: Hbptsc: counteranion.***

3.1. Antimicrobial assay

The results of the antibacterial assay of the copper (II) complexes of the ligand, ***Hbptsc*** have been shown in Table.1. The assay was carried out following the Agar-well diffusion method. Petriplates containing 20 ml Muller Hinton Agar Medium was seeded with bacterial culture (growth of culture adjusted according to Mc Fards Standard, 0.5%). Wells of approximately 10mm were bored using a well cutter and, test samples of 25 and100 µg/ml concentrations in DMSO were added. The plates were then incubated at 37°C for 24

hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well [20] using streptomycin as the positive control. Two Gram positive and two Gram negative strains were chosen. Out of four strains chosen at two different concentrations, [Cu(bptsc)NO_3] was found to be active against all strains of bacteria and showed largest inhibition zone of 3.0 and 3.5cm respectively at 25 and 100µg/ml for E.coli . The copper(II) complexes exhibit the following trend of antibacterial activity; [Cu(bptsc)NO_3]. > [Cu(bpstc)NCS]= [Cu(bptsc)Cl] >[Cu(bptsc)SO_4 >[Cu(bptsc)N_3]

Fig.3 shows the inhibition zones produced by control as well as E.coli for the complex Cu(bptsc)NO_3. The mode of action of complexes may be understood by the fact that ligands on chelation with the metal ions especially copper(II) show a reduction in the polarity thereby causing higher degree of toxicity to the cell membrane and cell walls of bacteria.[21, 22]. Besides chelation, higher toxicity of the metal complexes could be attributed to the involvement of metal ion in the normal cell process [23]. The metal ion interaction is found to be the maximum at the cell walls and membranes made up of lipids and polysaccharides. A decrease in polarity further increases the lipophilic character of the chelate favouring the interaction between the metal ion and the lipid. As a result the permeability barrier of the cell is broken down interfering with the normal cell processes. The geometry and charge distribution around the molecule also plays a vital role in determining teh effieciency of activity against the bacteria [24]. The presence of polar substituent's and anion coordination also adds to the antibacterial activity [25].

Fig.3 Petriplate showing inhibition zones produced by control as well as E.coli for the complex Cu(bptsc)NO_3

Table.1: The inhibition zones of the copper (II) complexes (in cm's) at two different concentrations (µg/ml)

Bacterial culture	[Cu(bptsc)Cl]..		[Cu(bptsc)(NO_3)]		[Cu_2(bptsc)$_2$$(SO_4)$].2$H_2O$		[Cu(bptsc)N_3]		[Cu(bptsc)(NCS)]	
Gram positive	25 µg/ml	100µg/ml	25 µg/ml	100 µg/ml	25 µg/ml	100 µg/ml	25µg/ml	100 µg/ml	25 µg/ml	100 µg/ml
Staphylococcus aureus	1.4	2.0	1.3	2.0	-		-	1.4	3.1	3.6
Streptococcus mutans	1.8	2.5	2.8	3.2	2.3	3.1	1.5	1.9	1.5	2.4
Gram negative										
Pseudomonas aeruginosa	-	1.5	1.0	1.6	-	1.3	-	1.4	1.0	1.7
Escheria coli	3.0	3.4	3.0	3.5	2.8	3.3	-	-	1.2	2.0

3.2. Short term *in vitro* cytotoxicity studies

The short term in vitro cytotoxicity studies of the copper (II) complexes have been shown in ***Table.2***. The studies was carried out following the trypan blue exclusion method. 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. viability of the cells was checked in a haemocytometer. The viable cells suspension (1×10^6 tumour cells/ 0.1ml PBS) were incubated in clean sterile tubes with the test compounds (25, 50, 100, 200 µg/ml in dimethyl sulfoxide (DMSO)) for 3h at 37°C, keeping the final volume at 1.0 ml. The control tube contained only cell suspension. The cell suspension was mixed with 0.1ml of 1%trypan blue and kept for 2-3 minutes 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. % Cytotoxicity = (No. of dead cells/No. of live cell+ No. of dead cell) ×10. 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].

Table.2: The short term in vitro cytotoxicity results of the copper (II) complexes.

Concentration	% cytotoxicity of complexes				
µg/ml	[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

REFERENCES

[1] V. Philip, V.Suni, M. R. P. Kurup, M. Nethaji, *Polyhedron*, 2006 (25) 1931-1938.

[2] V. Philip, V.Suni, M. R. P. Kurup, M. Nethaji, *Polyhedron*, 2004 (23) 1225-1233.

[3] Murugkar,A.;Padhye,S.;GuhaRoy,S.;Wagh,U.Inorg.Chem . Commun.1999,2, SinnE.; Fok, J.; Mehta, K.; Rath, N. Inorg. Chem. 2005, 44, 1154; (d) Kowol, C. R.;Trondl, R.; Heffeter, P.; Arion, V. B.; Jakupec, M. A.; Roller, A.; Galanski, M.;Berger, W.; Keppler, B. K. J. Med. Chem. 2009, 52, 5032.

[4] G. Domagk, R. Behnish, F. Mietzsch, H. Schmidt. New compound active against tuberculosis bacilli *invitro*. *Naturwissenschaften* 1946; 33:315.

[5] D. X. West, C. S. Carison, A. E. Liberta, J. N. Albert, C. R. Daniel. Transition metal ion complexes of thiosemicarbazones derived from2-acetylpyridine. Part 8. The chemical and antifungal properties of nickel(II) complexes of 2-acetylpyridine 4N-diethyl- and 4N-dipropylthiosemicarbazones and their copper (II) complexes. *Transit Met Chem* 1990;15: 341-4.

[6] A.E. Liberta, D.X. West, Biometals 5 (1992) 12

[7] D.L. Klayman, J.F. Bartoserich, T.S. Griffin, C.J. Mason, J.P. Scovill, *J. Med. Chem*. 22 (1979) 885[8]

[8] D.X. West, J.S. Billeh, J.P. Jasinski, J.M. Jasinski, R.J. Butcher, *Trans. Met. Chem*. 23 (1998) 209.

[9] Z. Afrasiabi, E. Sinn, S. Padhye, S. Dutta, S. Padhye, C. Newton, C.E. Anson, A.K..Powell, J. Inorg. Biochem. 95 (2003) 306.

[10] B. Atasever, B. Ulkuseven, T. Bal-Demirci, S. Erdem-Kuruca, Z. Solakog˘lu, Invest, New Drugs 28 (4) (2010) 421.

[11] M. Karatepe, F. Karatas, Cell. Biochem. Funct. 24 (2006) 547.

[12] R. Yanardag, T. Bal-Demirci, B. Ulkuseven, S. Bolkent, S. Tunalı, S. Bolkent, Eur.J. Med. Chem. 44 (2009) 818.

[13] A.E. Liberta, D.X. West, Biometals 5 (1992) 121.

[14] A.E. Graminha, A.A. Batista, J. Ellena, E.E. Castellano, L.R. Teixeira, I.C. Mendes,H. Beraldo, J. Mol. Struct. 875 (1–3) (2008) 219.

[15] T.S. Lobana, G. Bawa, A. Castineiras, R.J. Butcher, Inorg. Chem. Commun. 10 (2007) 506.

[16] I.C. Mendes, L.R. Teixeira, R. Lima, H. Beraldo, N.L. Speziali, D.X. West, J. Mol.
Struct. 559 (2001) 355.

[17] S. Adsule, V. Barve, D. Chen, F. Ahmed, Q.P. Dou, S. Padhye, F.H. Sarkar, J. Med Chem. 49 (2006) 7242.

[18] M. Sebastian, V. Arun, P.P. Robinson, A.A. Varghese, R. Abraham, E. Suresh, K.K.M. Yusuf Polyhedron 29 (2010) 3014.

[19] S.A. Filimon, C.G. Hrib, S. Randoll, I. Neda, P.G. Jones, M. Tamm, Z. Anorg, Allg. Chem. 636 (2010) 691.

[20] Copper and Its Complexes in Medicine: A Biochemical Approach,Isidoros Iakovidis, Ioannis Delimaris, and StylianosM. Piperakis, *Review Article*

[21] J.P.Scovill,,Phosphorous, Sulfur. Silicon Relat.Elem.60 (1991) 321

[22] P. Bindu ,MRP Kurup ,Indian J Chem 1997;36A:1094

[23] Saraswati, R. Dinda, C.S. Schmiesing, E. Sinn,Y.P. Patil, M. Nethaji, H. Stoeckli-Evans,R.Acharyya,Mixedligandnickel(II)thiosemicarbazonecomplexes:synthesis, characterization and biological evaluation, Polyhedron 50 (2013) 354.[21] Thangadurai T D, Natarajan K. Synthesis and characterization of new ruthenium(III) complexes containing tetradentate Schiff bases. *Syn. React. Inorg. Met. Org. Chem.*, 2001, **31**: 549.

[24] Srivastava R S. Pseudotetrahedral Co(II), Ni(II) and Cu(II) complexes of N1-(o-chlorophenyl)-2-(2′,4′-dihydroxyphenyl)-2-benzylazomethine their fungicidal and herbicidalactivity. *Inorg. Chim. Acta*, 1981, **56**: 65.

[25] Chakrabarti P, Anion binding sites in protein structures. *J.Mol. Biol*., 1993, **234**: 463.

[26] National Committee for Clinical Laboratory Standards. (1993a). Performance Standards for Antimicrobial Disk Susceptibility Tests—Fifth Edition: Approved Standard M2-A5. NCCLS, Villanova, PA.

[27] K.M.J. Swanson, F.F. Busta, E.H. Peterson, M.G. Johnson, Colony count methods,in: C. Vanderzant, D.F. Splittstoesser (Eds.), Compendium of Methods forMicrobiological Examination of Foods, third ed., American Public HealthAssociation, Washington DC,1992, pp. 75–95.

[28] A.M.Ramadan, Structural and biological aspects of copper (II) complexes with2-methyl- 3-amino-(3 H)-quinazolin-4-one, J. Inorg. Biochem. 65 (1997) 183.
J. Long, Inorg.Chem. 1968,**7,** 3401.