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## Revised Manuscript

# Synthesis, Structures, Antimicrobial Activity and Biosafety Evaluation of Pyridine-2-formaldehyde-N-susbtituted-thiosemicarbazonates of copper(II) †

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Pyridine-2-formaldehyde-$N^1$-substituted-thiosemicarbazones {$(C_5H_4N^4)HC^2{=}N^3{-}N^2(H)$ - $C^1$ = (S) $N^1HR$} (abbrev. $HL^1$-R : R = Me, Et, Ph) with copper(I) halides in acetonitrile-dichloromethane mixture (1:1, v/v) have yielded a novel series of $Cu^{II}$ complexes with central coordination core : [$Cu^{II}X(N,N,S\text{-}L^1\text{-}R)$] (R = Me, Et, Ph : X = I, **1-3**; Br, **4-6**, Cl, **7-9**), which have been characterized with the help of analytical data, IR, UV-visible and ESR spectroscopy, ESI-mass spectrometry and single crystal x-ray crystallography. The thio-ligands are coordinating to $Cu^{II}$ as anions $(L^1\text{-}R)^-$ through pyridyl nitrogen- $N^4$, azomethine nitrogen-$N^3$ and thiolato sulfur and $Cu^{II}$ is further bonded to iodide, bromide or chloride. In order to explore their bio-activity, complexes (**1-9**) as well as the previously reported $Cu^{II}$ complexes of 2-benzoylpyridine-N-substituted thiosemicarbazones {$(C_5H_4N^4)(C_6H_5)\text{-}C^2{=}N^3{-}N^2(H){-}C^1{=}(S)\text{-}N^1HR$; $HL^2$-R}, namely, [$Cu^{II}X(N,N,S\text{-}L^2\text{-}R$] (R = Me, Et, Ph : X = I, **10-12**; Br, **13-15**; Cl, **16-18**), have been evaluated for their antimicrobial potential against different microbial strains. The microbial strains investigated are, *Staphylococcus aureus* (MTCC740), *Klebsiella pneumonia* (MTCC530), *Shigella flexneri* (MTCC1457), *Salmonella typhimurium* 1 (MTCC98), *Salmonella typhimurium* 2 (MTCC1251), *Escherichia* coli (MTCC119), *Staphylococcus epidermidis* (MTCC435), methicillin resistant *Staphylococcus aureus* (MRSA) and a yeast culture *Candida albicans* (MTCC227). Several complexes tested have shown high antimicrobial activity; specifically, against methicillin resistant *Staphylococcus aureus, Staphylococcus epidermidis* and *Salmonella typhimurium* 2, the activity is found to be high at low minimum inhibitory concentration (MIC) values as compared to that of the standard Gentamicin. Using MTT cytotoxicity assay, {MTT = 3-

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[(4,5-dimethylthiazol-2-yl)-2,5-diphenyl] tetrazolium bromide}, complexes tested were found to be biosafe with high cell viability (90-98%).

**Key words :** Pyridine based thiosemicarbazones; Molecular structures, Copper(II), Antimicrobial agents; Viable cell count studies; MTT assay.

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## 1 Introduction

Thiosemicarbazones, ($R_1R_2C^2=N^3-N^2(H)-C^1(=S)N^1R_3R_4$}, are an interesting class of thio-ligands in which variation of R groups at the $C^2$- and $N^1$- atoms provide variable coordination activity towards different metals. The structural diversity of metal complexes of thiosemicarbazones including cyclometallation, biochemical activity and analytical applications are some of the key areas which have invited interest of the several research workers.[1-12] Metal derivatives of pyridine based thiosemicarbazones comprise an important class of compounds which have shown promising anticancer, antitumor and anti- tuberculosis activity,[13-29] as well as, antimicrobial activity.[30-38] Chart 1 shows different types of pyridine based thio-ligands, whose metal complexes have been investigated in the literature[13-38] for their bio-chemical activity. The ligands of types **A,**[13, 15-17, 20-29, 36] **B**,[14, 18, 19], and **C**[13] formed complexes with Cu,[13-19] Au,[26-28] Pd-Pt,[22-25] Zn-Cd,[20,21] and Fe[29] which have shown significant anticancer, antitumor and anti-

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mycobacterium tuberculosis activity. The antimicrobial activity of complexes of thio-ligands **D**,[30] **E** [38] and **A** [31-37] type with Cu,[30- 33] Ni,[34, 35] Pd-Pt, [36, 37] Mn,[38] Fe,[38] Cr [38] and Zn [38] has also been reported, but studies are limited.

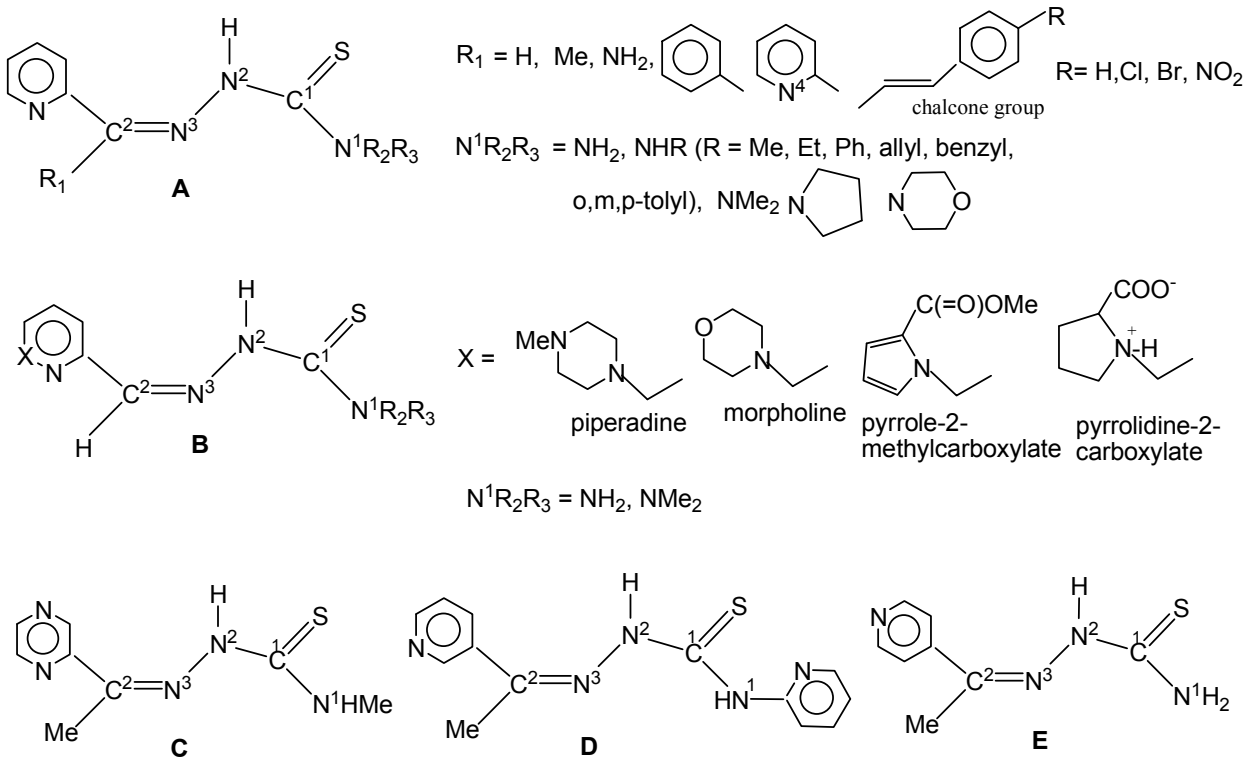


**Chart 1.**

**Chart 1**. Pyridine based thiosemicarbazones as ligands (A, B); other related pyridine type ligands (C, D, E)

From our laboratory, coordination chemistry of copper(I) halides with pyridine-2-formaldehyde thiosemicarbazones ($HL^1$-R, R = H, Me, Et, Ph) and 2-benzoylpyridine thiosemicarbazones ($HL^2$-R, R = H, Me, Et, Ph) (**Chart 2** for $HL^1$-R and $HL^2$-R) has been reported.[39-44] It was found that copper(I) halides with the thio-ligands $HL^1$-R (R = H, Me, Et and Ph) and $PPh_3$ in 1 : 1 : 2 (Cu : thio-ligand : $PPh_3$) molar ratio in acetonitrile yielded mononuclear complexes, $[CuX(\kappa S^1\text{-}HL^1\text{-}R)(PPh_3)_2]$ (X = Cl, Br, I).[39, 40] Similarly, the thio-ligand, $HL^2$-R (R = H) with copper(I) halides and $PPh_3$ in 1 : 1 : 2 (Cu : thio-ligand : $PPh_3$) molar ratio in acetonitrile-dichloromethane mixture formed mononuclear complexes, $[CuX(\kappa S^1\text{-}HL^2\text{-}$

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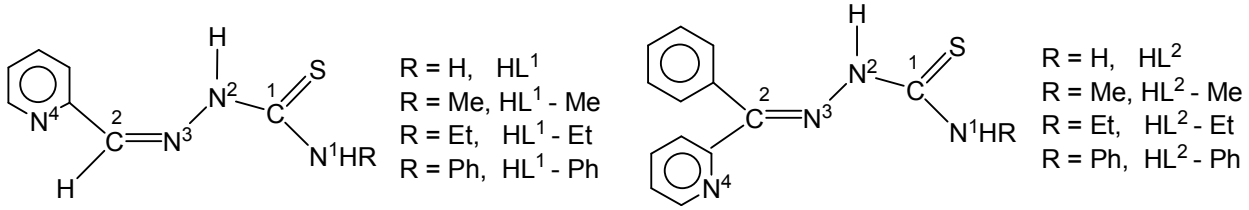
R)($PPh_3$)$_2$] (X = Cl, Br; R = H).[42] When copper(I) halides were reacted first with $PPh_3$ in acetonitrile followed by the addition of $HL^1$-R in chloroform in 1 : 1 : 1 molar ratio (for R = H; Cu : $PPh_3$ : thio-ligand), the products were the formation of unusual polymers, namely, $[Cu_4X_4(\mu_3\text{-S}, N^4\text{-}HL^1\text{-}R)_2(PPh_3)_4]_n$ (R = H; X = Cl, Br), a pyridine protonated product, $[CuBr_2(\eta^1\text{-S-}HL^1\text{-}R)^+(PPh_3)]$ (R = H) and an iodo-bridged dimer, $[Cu_2(\mu\text{-I})_2(\kappa S^1\text{-}HL^1\text{-}R)_2(PPh_3)_2]$ (R = H).[41]

Interestingly, reactions of copper(I) halides with 2-benzoylpyridine thiosemicarbazones ($HL^2$-R, R = H, Me, Et, Ph) (Chart 2) in acetonitrile-dichloromethane mixture (1:1, v/v) yielded copper(II) complexes of stoichiometry, [CuX(N,N,S-$L^2$-R] {$L^2$-R is anion with R = Me, Et, Ph : X = I, **10-12**; Br, **13-15**; Cl, **16-18**}, via proton coupled electron transfer (PCET) mechanism.[44] In this investigation, we report new $Cu^{II}$ complexes, [CuX(N,N,S-$L^1$-R)] (**1-9**), obtained from reactions of copper(I) halides with pyridine-2-formaldehyde thiosemicarbazones ($HL^1$-Me, $HL^1$-Et and $HL^1$- Ph, structure I, Chart 2) in $CH_3CN$-$CH_2Cl_2$ mixture, on the pattern of 2-benzoylpyridine thiosemicarbazones.[44]




I . Thio-ligands, $HL^1$- R

II. Thio-ligands, $HL^2$ - R

Chart 2.The thio-ligands under study in present investigation

Further, owing to our interest to investigate biochemical activity of coordination compounds based on thiosemicarbazones, recently, antimicrobial activity of $Cu^{II}/Zn^{II}$ complexes with salicylaldehyde–N-substituted thiosemicarabzones was reported.[45-49] These compounds showed bactericidal activity but in vitro cell viability was low, precluding their further use as biosafe antimicrobial agents. In continuation, due to the biochemical importance of complexes of copper(II) with pyridine based thiosemicarbazones as outlined above,[13-38] it was designed to explore new complexes **1-9** as well as previously reported complexes **10-18**[44] for evaluating their antimicrobial activity. It is highlighted here that the studied microorganisms cause a variety of infections and become resistant to various types of antibiotics and thus pose a continuous challenge to the researcher to look for alternative antimicrobial agents. For example, methicillin

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resistant *Staphylococcus aureus* (MRSA) is a resistant form of *Staphylococcus aureus.*[45-49] Thus, this paper has two fold objective, to report new $Cu^{II}$ complexes **1-9** of pyridine-2-carbaldehyde thiosemicarbazones ($HL^1$-R, R = H, Me, Et, Ph) and to investigate antimicrobial activity of these and previously reported complexes **10-18** of 2-benzoylpyridine thiosemicarabzones.[44]

In respect of copper(II), the metal under study, it was noted from the literature that there were a few reports as regards antimicrobial activity of complexes of A[31-33] or D[30] type ligands (Chart 1). The antimicrobial studies (*E.coli*, P.*aeruginosa*,[30] *Salmonella typhimurium*, *Candida albicans*,[31,32] *vibrio cholerae* O1,[33]) reported are limited to zone of inhibition or minimum inhibitory concentration and there are no reports about viable cell count studies through time kill assay and their bio-safety tested by MTT assay. The current investigation reports synthesis of new $Cu^{II}$ complexes, and antimicrobial activity of complexes (**1-18**) in terms of zone of inhibition, minimum inhibitory concentration (MIC), viable cell count studies through time kill assay and their bio-safety tested by MTT assay. Several significant microbial strains not studied earlier are investigated.

## 2 Experimental section

### 2.1. Chemicals and techniques

Pyridine-2-formaldhyde, thiosemicarbazide, N-methylthiosemicarbazide, N-ethylthiosemicarbazide, N-phenylthiosemicarbazide were purchased from Aldrich Sigma Ltd. Thio-ligands were prepared by refluxing a thiosemicarbazide with pyridine-2-formaldehyde in presence of acetic acid in methanol for a period of 24 h (see supporting information for details). Copper(I) halides were freshly prepared by the reduction of $CuSO_4 \cdot 5H_2O$ with alkali halide by passing over $SO_2$ gas through the solution.[50] Elemental analysis (C, H, N, S) were carried out using the THERMO FINNIGAN FLASH technique. Melting points were determined with a Gallenkamp electrically heated apparatus. The IR spectra were recorded using KBr pellets with a VARIAN FT-IR 670 spectrophotometer in the 4000–400 $cm^{-1}$ range. The ESI-mass spectra were recorded in DMSO using a Bruker Daltonik LS-MS high resolution micro TOF-Q II 10356. The UV-visible spectra of the compounds were recorded in DMSO with the help of a UV-1601 PC Shimadzu spectrophotometer.

### 2.2. Synthesis of complexes

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**[CuI(N,N,S-L$^1$-Me)] 1.** To a pale yellow solution of copper(I) iodide (0.025 g, 0.13 mmol) in acetonitrile (15 mL), solid ligand HL$^1$-Me (0.025 g, 0.13 mmol) was added. To it 10 mL of dichloromethane was added and the solution was stirred for one day during which the color of the solution starts changing to dark green. After this the contents were refluxed for 30 minutes and filtered. Slow evaporation of the solution yielded a dark green crystalline product in 2- 3 days. Analytical data supported the formation of complex, [Cu$^{II}$($\kappa^3$-N,N,S-L$^1$-Me)I]. Yield : 0.031 g, 62%, m.p. 180-182ºC. Anal.Calc. for $C_8H_9CuIN_4S$: C, 25.03; H, 2.35; N, 14.60; S, 8.34 ; found: C, 24.89; H, 2.47; N, 14.30; S, 8.09 . Main IR bands (KBr, cm$^{-1}$): ν(N$^1$–H) 3212s; ν(C–H) 3013m, 2970m, 2932m; ν(C=N) + ν(C=C) + δ(N−H), 1632w,1593m, 1568m, 1532s; δ(C–H) + ν(C−N) 1472m, 1441w, 1351s, 1304m; other bands, 1281m, 1225m, 1176m, 1157m, 1118m, 1101m, 1041m; ν(C−S) 882m; other bands, 859m, 770m, 740w, 690w, 646w, 608m, 516w, 478m. Electronic absorption spectrum, DMSO, $\lambda_{max}$/nm, ε/L mol$^{-1}$cm$^{-1}$: [10$^{-3}$ M] 627 (1.33 × 10$^2$); [10$^{-4}$M] 296 (1.214 × 10$^4$), 343 (0.957 × 10$^4$), 403 (1.052 × 10$^4$). This complex has low solubility in dichloromethane, methanol and acetonitrile, but is soluble in DMSO and DMF. Complexes **4, 7, 8** and **9** were also prepared by this method.

**[Cu$_2$I$_2$($\kappa^4$: N,N,μ-S-L$^1$-Et)$_2$] 2.** To a pale yellow solution of copper(I) iodide (0.025 g, 0.13 mmol) in acetonitrile (15 mL), solid ligand HL$^1$-Et (0.027 g, 0.13 mmol) was added. The solution was stirred for one hour during which the color of the solution starts changing to dark green. After this, the solution was refluxed for ten minutes and a dark green solid was formed. The solid was dissolved in dichloromethane (8-10 mL) and the solution was left for crystallization at room temperature. Crystals were formed after a period of 2-3 days. Analytical data supported the formation of the complex, [Cu$_2^{II}$($\kappa^4$: N,N,μ-S-L$^1$-Et)$_2$I$_2$]. Yield: 0.027 g, 52%, m.p. 181-183ºC. Anal. calc. for $C_9H_{11}CuIN_4S$ : C, 27.17; H, 2.77; N, 14.09; S, 8.05; found: C, 27.59; H, 2.78; N, 14.23; S, 8.03. Main IR bands (KBr, cm$^{-1}$): ν(N$^1$–H) 3381s, 3339m; ν(C–H), 3035w, 2971w, 2930w; ν(C=N) + ν(C=C) + δ(N−H) 1604s, 1558m,1495s, 1433s; δ(C–H) + ν(C−N) 1358m, 1335m; other bands, 1266w, 1230s, 1150s, 1052w, 1016w, 969m; ν(C−S) 903m; other bands, 774s, 635m, 515m, 483m, 417m. Electronic absorption spectrum, DMSO, $\lambda_{max}$/nm, ε/L mol$^{-1}$cm$^{-1}$: [10$^{-3}$ M] 626 (1.33 × 10$^2$); [10$^{-4}$M] 307 (3.254 × 10$^4$), 338 (2.323 × 10$^4$),

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405 ($2.532 \times 10^4$). Complexes **3, 5** and **6** were prepared by this method. Crystals of each of **2**, **3**, **5** and **6** were grown from a acetonitrile-dichloromethane mixture.

**$[Cu_2I_2(\kappa^3$:N,N,S-$L^1$-Ph)($\kappa^4$:N,N,μ-S-$L^1$-Ph)]·¼$CH_2Cl_2$ 3.**Yield: 0.030 g, 52%,m.p. 141-143ºC. Anal. calc. for $C_{13}H_{11}CuIN_4S·¼CH_2Cl_2$ : C, 34.06 ; H, 2.46; N, 12.00; S, 6.86 ; found: C, 33.83; H, 2.54; N, 12.07; S, 7.05 . Main IR bands (KBr, $cm^{-1}$): ν($N^1$–H) 3315w, 3282w ; ν(C–H) 3058m, 2954w, 2810w; ν(C=N) + ν(C=C) + δ(N−H) 1596s, 1546s, 1495s; δ(C–H) + ν(C−N) 1454s, 1433s, 1355m, 1313s; other bands, 1230s, 1127s, 1100w,; ν(C−S) 908 m; other bands, 828w, 751s, 691s, 583m, 503m. Electronic absorption spectrum, DMSO, $\lambda_{max}$/nm, ε/L $mol^{-1}cm^{-1}$: [$10^{-3}$ M] 680 ($0.25 \times 10^2$); [$10^{-4}$M] 274 ($1.812 \times 10^4$), 346 ($0.922 \times 10^4$), 407 ($0.834 \times 10^4$). This complex has low solubility in dichloromethane, methanol and acetonitrile, but is soluble in DMSO and DMF.

**[CuBr(N,N,S-$L^1$-Me)] 4.** Yield: 0.028 g, 47.5%, m.p. 192-196ºC. Anal. Calc. for $C_8H_9BrCuN_4S$: C, 28.53; H, 2.67; N, 16.64; S, 9.51; Found: C, 28.35; H, 2.47; N, 16.48; S, 9.54. Main IR bands (KBr, $cm^{-1}$): ν($N^1$–H) 3222s; ν(C–H) 3086m, 3016m, 2977m, 2937m; ν(C=N) + ν(C=C) + δ(N−H) 1593s, 1571s, 1533s, 1470m; δ(C–H) + ν(C−N) 1440m, 1356s, 1305s; other bands, 1283s, 1228m, 1181m, 1155w, 1118m, 1098w, 1041s; ν(C−S) 898m; other bands, 883w, 861m, 823w, 777m, 742w, 721w, 707w, 690w, 645m, 607m, 520m, 490m. Electronic absorption spectrum, DMSO, $\lambda_{max}$/nm, ε/L $mol^{-1}cm^{-1}$: [$10^{-3}$ M] 627 ($0.71 \times 10^2$); [$10^{-4}$M] 300 ($0.786 \times 10^4$), 348 ($0.546 \times 10^4$), 407 ($0.653 \times 10^4$). This complex has low solubility in dichloromethane, methanol and acetonitrile, but is soluble in DMSO and DMF.

**$[Cu_2Br_2(\kappa^4$:N,N,μ-S-$L^1$-Et)$_2$] 5.** Yield: 0.022 g, 36%, m.p. 166-168ºC. Anal. Calc. for $C_9H_{11}BrCuN_4S$ : C, 30.81; H, 3.14; N, 15.98; S, 9.12. Found, C, 30.80; H, 3.05; N, 15.84; S, 9.04; Found: Main IR bands (KBr, $cm^{-1}$): ν($N^1$–H) 3315s; ν(C–H), 3093w, 2974m, 2928w, 2872w; ν(C = N) + ν(C=C) + δ(N−H) 1604m, 1565m, 1509s, 1484m; δ(C–H) + ν(C−N)1440s, 1360w, 1333w; other bands, 1300w, 1261w, 1235s, 1148s, 1100w, 1049w; ν(C−S) 882m; other bands, 774s, 669w, 636m, 546m, 453w, 416m. Electronic absorption spectrum, DMSO, $\lambda_{max}$/nm, ε/L $mol^{-1}cm^{-1}$: [$10^{-3}$ M] 620 ($1.31 \times 10^2$); [$10^{-4}$M] 299 ($3.545 \times 10^4$), 333 ($2.78 \times 10^4$), 407

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($2.706 \times 10^4$). This complex has low solubility in dichloromethane, methanol and acetonitrile, but is soluble in DMSO and DMF.

**$[Cu_2Br_2(\kappa^3$:N,N,S-$L^1$-Ph)($\kappa^4$:N,N,μ-S-$L^1$-Ph)] 6.** Yield: 0.023 g, 34%, m.p. 138-140ºC. Anal. Calc. for $C_{13}H_{11}BrCuN_4S$: C, 39.15; H, 2.76; N, 14.05; S, 8.03; Found: C, 38.74; H, 2.89; N, 13.87; S, 8.31. Main IR bands (KBr, $cm^{-1}$): ν($N^1$–H) 3319w, 3273w; ν(C–H) 3181w, 3061w; ν(C=N) + ν(C=C) + δ(N−H) 1596s, 1550s, 1494s; δ(C–H) + ν(C−N) 1434s, 1357m, 1316m; other bands, 1249m, 1157w, 1127m,1076w, 1028w; ν(C−S) 910m; other bands, 831w 753s, 692s, 610w, 494m. Electronic absorption spectrum, DMSO, $\lambda_{max}$/nm, ε/L $mol^{-1}cm^{-1}$: [$10^{-3}$ M] 615 ($1.72 \times 10^2$); [$10^{-4}$M] 304 ($2.025 \times 10^4$), 352 ($2.424 \times 10^4$), 401 ($3.085 \times 10^4$). This complex has low solubility in dichloromethane, methanol and acetonitrile, but is soluble in DMSO and DMF.

**$[CuCl(N,N,S\text{-}L^1\text{-}Me)]\cdot\frac{1}{2}CH_2Cl_2$ 7.** Yield: 0.035 g, 47%, m.p. 188-190ºC. Anal. Calc. for $C_8H_9ClCuN_4S\cdot\frac{1}{2}CH_2Cl_2$ : C, 30.49; H, 2.98; N, 16.74; S, Found: C, 30.72; H, 2.99; N, 17.03. Main IR bands (KBr, $cm^{-1}$): ν($N^1$–H) 3216s; ν(C–H) 3170w, 3089w, 3016m, 2976s, 2836m, 2890w; ν(C=N) + ν(C=C) + δ(N−H) 1594s, 1571m, 1537s, 1471s, 1441m; δ(C–H) + ν(C−N) 1356s, 1307s; other bands, 1284s, 1226m, 1182m, 1155w, 1122s, 1099m, 1044s; ν(C−S) 900w, 883w; other bands, 859w,778m; 727w, 692w, 646w, 610m, 521m, 483m, 416m. Electronic absorption spectrum, DMSO, $\lambda_{max}$/nm, ε/L $mol^{-1}cm^{-1}$: [$10^{-3}$ M] 627 ($1.00 \times 10^2$); [$10^{-4}$M] 296 ($0.802 \times 10^4$), 340 ($0.636 \times 10^4$), 408 ($0.689 \times 10^4$). This complex has low solubility in dichloromethane, methanol and acetonitrile, but is soluble in DMSO and DMF.

**$[CuCl(\kappa^3$: N,N,S-$L^1$-Et)]$\cdot\frac{1}{2}CH_3CN$ 8.** Yield : 0.034 g, 44%, m.p. 178-180ºC. Anal. Calc. for $C_9H_{11}ClCuN_4S\cdot\frac{1}{2}CH_3CN$: C, 36.75; H, 3.83; N, 19.29; S, 9.80; found C, 36.65; H, 3.75; N, 19.17; S, 10.17. Main IR bands (KBr, $cm^{-1}$): ν($N^1$–H) 3322s; ν(C–H) 3064w, 2977w, 2932w, 2875w; ν(C=N) + ν(C=C) + δ(N−H) 1604m, 1584w, 1561w, 1512s, 1485m, ; δ(C–H) + ν(C−N) 1437s, 1387w, 1359w, 1334w; other bands, 1236s, 1149s, 1036s, 1097s; ν(C−S) 903w, 884m; other bands, 799w, 778m; 744w, 669w, 638w, 517w, 472m, 415w. Electronic absorption spectrum, DMSO, $\lambda_{max}$/nm, ε/L $mol^{-1}cm^{-1}$: [$10^{-3}$ M] 630 ($1.73 \times 10^2$); [$10^{-4}$M] 297 ($1.38 \times 10^4$),

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351 (0.123 × $10^4$), 417 (1.247 × $10^4$). This complex has low solubility in dichloromethane, methanol and acetonitrile, but is soluble in DMSO and DMF.

**[CuCl(N,N,S-$L^1$-Ph)]·¼$CH_2Cl_2$ 9.**Yield : 0.039 g, 44%, m.p. 210-213ºC. Anal. Calc. for $C_{13}H_{11}ClCuN_4S$ : C, 44.07; H, 3.11; N, 15.82; S, 9.04; Found: C, 43.72; H, 3.21; N, 15.69; S, 8.86. Main IR bands (KBr, $cm^{-1}$): ν($N^1$–H) 3348s, 3292w; ν(C–H) 3128w, 3072w; ν(C=N) + ν (C=C) + δ(N−H) 1598s, 1542s, 1491s, 1455s; δ(C–H) + ν(C−N) 1428s, 1355m, 1321m; other bands, 1249m, 1218m, 1188m, 1131s; 1100m; ν(C−S) 896m; other bands, 833w, 752m, 692m, 671w, 641w, 589w, 471m.T Electronic absorption spectrum, DMSO, $\lambda_{max}$/nm, ε/L $mol^{-1}cm^{-1}$: [$10^{-3}$ M] 613 (1.89 × $10^2$); [$10^{-4}$M] 306 (0.957 × $10^4$), , 358 (1.09 × $10^4$), 415 (1.292 × $10^4$). This complex has low solubility in dichloromethane, methanol and acetonitrile, but is soluble in DMSO and DMF.

### 2.3 X-ray crystallography

The data for complexes **2** and **3** were collected at 100(2) and 296(2) K respectively on Bruker APEX-II CCD X-ray diffractometer equipped with a graphite monochromator and Mo–Kα radiation (λ = 0.71073 Å). The structures were solved by the direct methods using SIR-92 and it was refined by the least square methods on $F^2$ using SHELXL-97 program. All atoms were refined anisotropically. Hydrogens were calculated geometrically.[51-53] The crystal structures of complexes **5** and **6** were determined by mounting a single crystal of a complex on a nylon loop and the data were measured with an Rigaku, Oxford Diffraction Eos, Gemini diffractometer, equipped with the graphite monochromated Mo-Kα (λ = 0.71073 Å) radiation at 173(2) K. The structures were solved by the direct methods and refined using full-matrix least-squares techniques based on $F^2$ using ShelXL-2014. In these structures, all the non-hydrogen atoms were refined anisotropically and the hydrogens have been fixed geometrically.[52, 54-57] Crystal quality was poor in case of complex **3**. Several attempts were made to improve the quality, but all results were same. Table 1 contains crystal data.

**Table 1** Crystallographic data for complexes **2**, **3**, **5**, **6** and **8**

| | **2** | **3** | **5** | **6** | **8** |
|---|---|---|---|---|---|

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| Empirical formula | $C_{18}H_{22}Cu_2I_2N_8S_2$ | $C_{26}H_{22}Cu_2I_2N_8S_2$ | $C_{18}H_{22}Cu_2Br_2N_8S_2$ | $C_{26}H_{22}Cu_2Br_2N_8S_2$ | $C_{11}H_{14}ClCuN_5S$ |
|---|---|---|---|---|---|
| *M* | 795.48 | 891.56 | 701.45 | 797.53 | 347.32 |
| *T* /K | 100(2) | 296(2 | 173(2) | 173(2) | 293(2) |
| Crystal system | Monoclinic | Triclinic | Monoclinic | Triclinic | Triclinic |
| Space group | $P2_1/c$ | *P*-1 | $P2_1/c$ | *P*-1 | *P*-1 |
| a(Å) | 14.484(2) | 11.371(2) | 14.1753(7) | 11.1903(5) | 8.5331(7) |
| b(Å) | 17.112(4) | 12.013(2) | 17.1735(6) | 11.6509(6) | 9.4698(7) |
| c(Å) | 10.824(5) | 13.163(2) | 10.5732(4) | 12.8056(7) | 9.5110(7) |
| α (º) | 90 | 110.740(6) | 90 | 109.996(5) | 96.619(6) |
| β (º) | 110.919(4) | 104.322(6) | 111.788(5) | 105.367(4) | 98.510(6) |
| γ (º) | 90 | 105.686(7) | 90 | 104.566(4) | 102.123(7) |
| V(Å$^3$) | 2505.9(13) | 1496.8(5) | 2390.04(19) | 1400.85(14) | 734.58(10) ) |
| *Z* | 4 | 2 | 4 | 2 | 2 |
| $D_{calcd}$ (g.cm$^{-3}$) | 2.109 | 1.978 | 1.949 | 1.891 | 1.570 |
| $\mu$ (mm$^{-1}$) | 4.352 | 3.655 | 5.322 | 4.553 | 5.049 |
| *F*(000) | 1528 | 860 | 1384 | 788 | 354 |
| Reflns collected | 43695 | 14802 | 30586 | 18500 | 4611 |
| Unique reflns. | 5775 | 5345 | 8213 | 9286 | 2792 |
| | ($R_{int}$= 0.0613) | ($R_{int}$=0.0417) | ($R_{int}$ = 0.0965) | ($R_{int}$=0.0356) | ($R_{int}$=0.0350) |
| Data/ restraints / parameters | 5775 /2/295 | 5345/2 /367 | 8213/0/291 | 9286/0/361 | 2792 /0/174 |
| Reflecs. with [*I*>2σ(*I*)] | 5369 | 3643 | 4771 | 6788 | 2373 |
| Final *R* indices | $R_1$ = 0.0216 | $R_1$ =0.0423 | $R_1$ = 0.0613 | $R_1$ = 0.0378 | $R_1$ = 0.0468 |
| [*I*>2σ(*I*)] | w$R_2$ = 0.0546 | w$R_2$ = 0.0957 | w$R_2$ = 0.0985 | w$R_2$ = 0.0701 | w$R_2$ = 0.1164 |
| Final *R* indices | $R_1$ = 0.0239 | $R_1$ = 0.0695 | $R_1$ = 0.1312 | $R_1$ = 0.0641 | $R_1$ = 0.0546 |

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| | | | | | |
|---|---|---|---|---|---|
| (all data) | w$R_2$ = 0.0560 | w$R_2$ = 0.1073 | w$R_2$ = 0.1229 | w$R_2$ = 0.0793 | w$R_2$ = 0.1248, |
| Largest diff. | 0.455 and | 0.868 and | 1.241 and | 0.760 and | 0.622 and |
| peak/ hole e.Å$^{-3}$ | −0.580 | −0.911 | −1.230 | −0.933 | −0.678 |



### 2.4 Antimicrobial studies

The details of antimicrobial techniques employed for studying metal complexes are placed in the supporting information. The investigations are made against methicillin resistant *Staphylococcus aureus* (MRSA), Gram positive bacteria: *Staphylococcus aureus* (MTCC-740), *Staphylococcus epidermidis* (MTCC-435), Gram negative bacteria: *Klebisella pneumoniae* (MTCC-530), *Escherichia coli* (MTCC-119), *Salmonella typhimurium* 1 (MTCC-98), *Salmonella typhimurium* 2 (MTCC-1251), *Shigella flexneri* (MTCC-1457), and one yeast strain, *Candida albicans* (MTCC-227). In relation to live subject statement it is submitted here that the study has been carried out as per Institutional Bioethics Committee and National Guidelines. It is further stated it did not involve any experimentation on live animals or Humans. For cell viability studies sheep blood was procured from City Slaughter House.

## 3 Results and discussion

### 3.1 Synthesis and IR Spectroscopy

Chart 3 depicts various complexes under present investigation. Recently, we found that the reactions of copper(I) halides with 2-benzoylpyridine thiosemicarbazones ($HL^2$-R, R = Me, Et, Ph) in the $CH_3CN$-$CH_2Cl_2$ mixture yielded dark green $Cu^{II}$ complexes with central cores as: $[Cu^{II}X(\kappa^3$:N,N,S- $L^2$-R)] {X = Cl, Br, I; $(L^2$-R)$^-$ is anion with R = Me, Et, Ph}(Chart **3b**, **10-18**). [44] These reactions demonstrated the formation of complexes with stable $Cu^{II}$-iodide as well as formation of $Cu^{II}$ –bromide/ chloride bonds from copper(I) halides when reacted with $HL^2$-R thio-ligands.[43, 44] The formation of the above complexes was explained in terms of a proton coupled electron transfer (PCET) mechanism. In this paper, the coordination activity of pyridine-

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2-carbaldehyde-$N^1$-substituted-thiosemicarbazones ($HL^1$-R, R = Me, Et, Ph) towards copper(I) halides in $CH_3CN$-$CH_2Cl_2$ mixture has led to the formation of analogous $Cu^{II}$ complexes with central coordination cores as: $[Cu^{II}X(\kappa^3: N,N,S\text{-}L^1\text{-}R)]$ (X = I, Br, Cl; R = Me, Et, Ph, **1-9**) as depicted in Chart **3a** via the PCET mechanism.[44] It is added here that when copper(II) chloride or bromide were reacted with $HL^1$-R ligands, same products were formed, because $Cu^{II}$ is reduced to $Cu^I$ by the addition of a thio-ligand and reaction then proceeds in the same way as it occurs in the direct reaction of a copper(I) halide with the thio-ligand under purview. It was observed earlier as well with the analogous $HL^2$-R ligands (Chart 2).[33, 44] Further it was found that identical products were formed under anaerobic conditions with the thio-ligands $HL^1$-R in this work and with the thio-ligands $HL^2$-R reported earlier.[44]

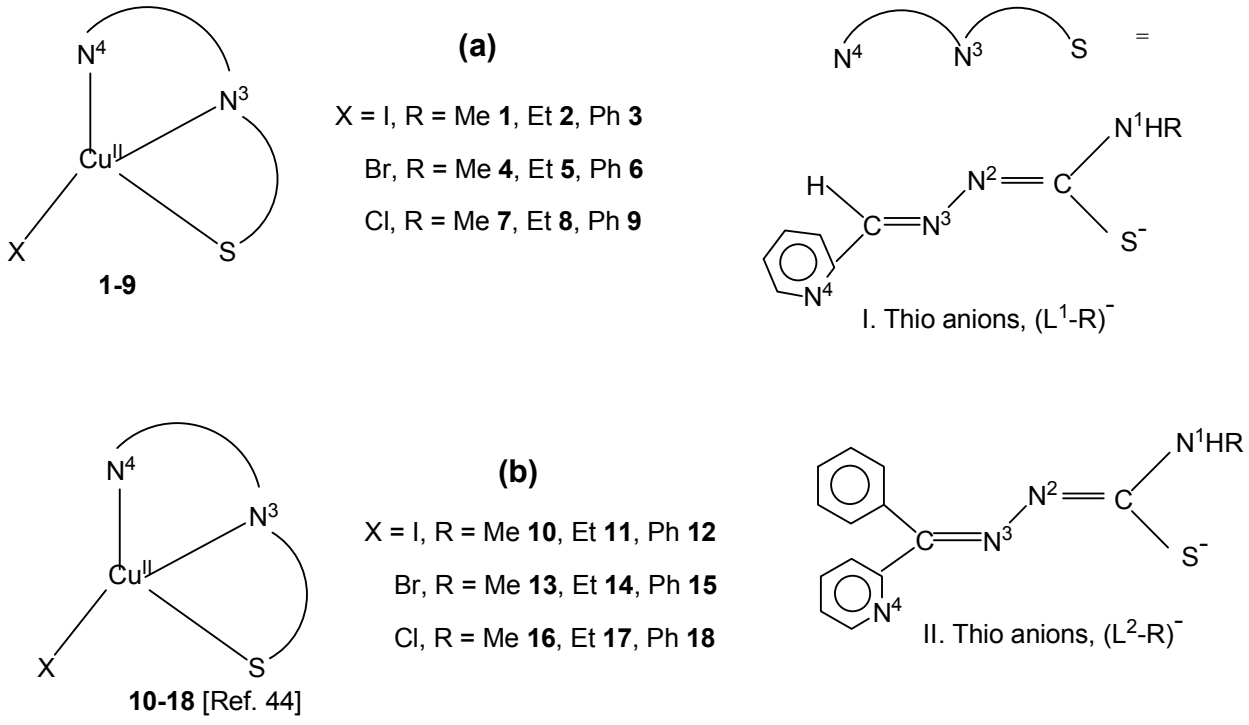


**Chart 3.**

Chart 3. Synthesis of $Cu^{II}$ complexes with in situ generated thio anions $(L^1\text{-}R)^-$

Various characteristic IR bands of these complexes are listed in the experimental section. The anionic nature of the coordinated thio-ligands, $(L^1\text{-}R)^-$, was supported by the disappearance of the $\nu(N^2\text{–}H)$ bands in the IR spectra of complexes, **1**–**9** [$\nu(N^2\text{–}H)$ : 3249-3260s $cm^{-1}$ for the

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free ligands, $HL^1$-R, R = Me, Et, Ph]. The ν(C–S) bands in these complexes are assigned in the region, 880 to 910 $cm^{-1}$ and vary in intensity from weak to strong in intensity. Various significant bands due to ν($N^1$-H), ν(C-H), δ(C-H), ν(C=N), ν(C=C), δ(N-H) and ν(C-N) are listed in the experimental section and the IR data overall serve as fingerprint for the respective complexes.

### 3.2 Molecular structures

Complexes **2** and **5** each crystallized in the monoclinic crystal system with the space group $P2_1/c$, while complexes **3, 6** and **8** each crystallized in the triclinic crystal system with space group P-1. Other complexes did not form suitable crystals for study using x-ray crystallography. The molecular structure of complex $\mathbf{2}_{(I, Et)}$ shows that one thio-ligand binds to Cu1 through its $N1_{(azomethine)}$, $N4_{(pyridyl)}$, S1 and I1 donor atoms with the bond distances of 1.9915(19), 2.023(2), 2.2731(7) and 2.5912(9) Å, respectively, comprising the coordination core, [$Cu^{II}I(\kappa^3$: N,N,S-$L^1$-Et)] (Fig. 1). The second thio-ligand binds to Cu2 through $N6_{(azomethine)}$, $N5_{(pyridyl)}$, S2 and I2 atoms at almost equal bond distances forming similar core . These two cores are linked through coordinated S1 and S2 donor atoms at long Cu1-S2 and Cu2-S1 bond distances of 2.7781(8) and 2.7085(8) Å, respectively, and thus the $Cu_2S_2$ core becomes a parallelogram. The angles around each of the Cu1 and Cu2 atoms lie in the range, 80 to 164° with trans S-Cu-$N_{(pyridyl)}$ angles, 163.59(6) {Cu1}, 163.67(6)° {Cu2} and trans $N_{(azomethine)}$-Cu-I bond angles, 159.49(6){Cu1}, 157.42(6)°{Cu2} and the angles within the $Cu_2S_2$ core are 84.62(3)° and 93.80(2)° at S1 and Cu1 respectively. The Cu-S distances of $Cu_2S_2$ core are longer than the usual Cu-S bond distances observed but are less than the sum of van der Waals radii of Cu and S (3.20 Å,[58]) and these distances indicate weak Cu···S interaction in the dinuclear complex **2**. The geometry around each metal center can be considered as distorted square pyramidal. The bonding pattern is similar to that found in the analogous complex, [$Cu^{II}I(\kappa^3$: N,N,S-$L^2$-Et)] (Chart 2 ).[44]

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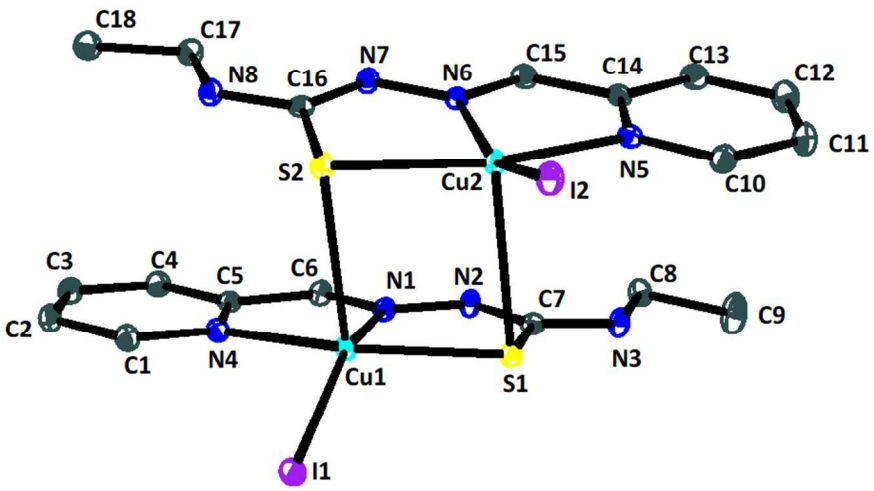


Fig. 1. ORTEP diagram of $[Cu_2^{II}I_2(\kappa^4: N,N,\mu\text{-}S\text{-}L^1\text{-}Et)_2]$ **2** with 30% ellipsoid probabilty.

Hydrogen atoms are omitted for more structure clarity.

The molecular structure of dimeric complex $[Cu_2^{II}Br_2(\kappa^4:N,N,\mu\text{-}S\text{-}L^1\text{-}Et)_2]$ $\mathbf{5}_{(Br,\ Et)}$ is shown in Fig. 2 and its bonding pattern is similar to that of complex $\mathbf{2}_{(I,\ Et)}$. In this complex, the bridging Cu-S bond distances are somewhat longer, while bonds in the square plane are similar to those found in complex **2**. The Cu-Br bond distances, 2.3962(7), 2.4013(7) Å, are somewhat longer than those found in an analogous complex, $[Cu^{II}Br(\kappa^3:N,N,S\text{-}L^2\text{-}Et)]$, having terdentate thio-anion,$(L^2\text{-}Et)^-$.[44] The geometry around each metal center is a distorted square pyramid. The distortion value of a coordination polyhedron is evaluated by the two largest bond angles in five coordination geometry using the relationship, $\tau = (\beta - \alpha)/60$ in which α and β are the two largest bond angles. The value of $\tau = 1$ is for an ideal trigonal bipyramid and $\tau = 0$ for square pyramidal geometry.[59] For complex $\mathbf{2}_{(I,\ Et)}$, the values of τ are 0.068, 0.104(Cu1, Cu2) and for complex $\mathbf{5}_{(Br,\ Et)}$, the values of τ are 0.051, 0.099 (Cu1, Cu2). These τ values suggest that geometry of each metal center is close to a square pyramid.

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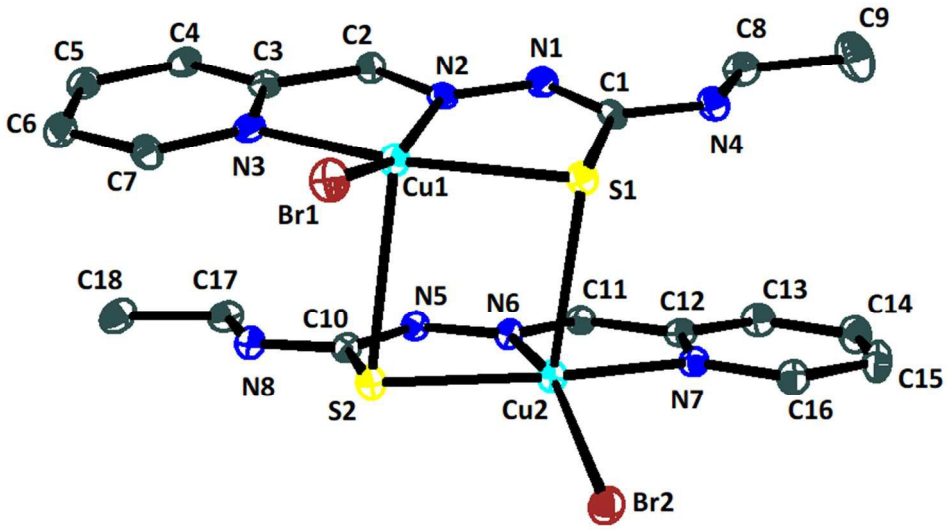


Fig. 2. ORTEP diagram of complex $[Cu_2^{II}Br_2(\kappa^4{:}N,N,\mu\text{-}S\text{-}L^1\text{-}Et)_2]$ **5** with 30% ellipsoid probabilty. Hydrogen atoms are omitted for more structure clarity.

In complex $[Cu_2^{II}I_2(\kappa^3{:}N,N,S\text{-}L^1\text{-}Ph)(\kappa^4{:}N,N,\mu\text{-}S\text{-}L^1\text{-}Ph)]$ **3,** (**3**$_{(I,\ Ph)}$), Cu1 is bonded to $N2_{(azomethine)}$, $N1_{(pyridyl)}$, S1 and I1 donor atoms of one thio-ligand with the bond distances of 1.979(5), 2.026(5), 2.2596(16) and 2.5569(8) Å, respectively, and the second thio-ligand binds to Cu2 through $N6_{(azomethine)}$, $N5_{(pyridyl)}$, S2 and I2 donor atoms at similar bond distances (Fig. 3) (See SI, Table S1). These Cu-S, Cu-$N_{(azomethine)}$ and Cu-$N_{(pyridyl)}$ and Cu-I bond distances, as well as the angles around each of the Cu1 and Cu2 atoms and trans S-Cu-$N_{(pyridyl)}$ and $N_{(azomethine)}$-Cu-I bond angles are similar to those of complex **2**$_{(I,\ Et)}$. Unlike double bridging observed in complexes **2**/**5**, there is a single bridge in dimer **3**$_{(I,\ Ph)}$, the donor atom S1 bridges Cu2 at long bond distance of 2.7993(19) Å which is similar to that found in **2**$_{(I,\ Et)}$. For this complex **3**, the value of τ is 0.042, which supports that the geometry around the Cu2 metal center is close to a square pyramid and that around Cu1 is distorted square planar. The bonding pattern is similar to that of complex $[Cu_2^{II}I_2(\kappa^3{:}N,N,S\text{-}L^1\text{-}Ph)(\kappa^4{:}N,N,\mu\text{-}S\text{-}L^1\text{-}Ph)]$ **3.** Here Cu1 is five coordinated and Cu2 is four coordinated. The Cu1-S2-Cu2 bond angle of 85.03(2)$^o$ as well as the value of τ = 0.028 confirm that the geometry of the Cu1 metal center is distorted square pyramid, like that of Cu2 metal center of analogous complex **3**. Molecular structure of complex **8** is shown in Fig. 4 and geometry is distorted square planar with bond parameters analogous those reported in literature for analogous complexes.[44] (see supporting information for bond parameters for all complexes; Table S1).

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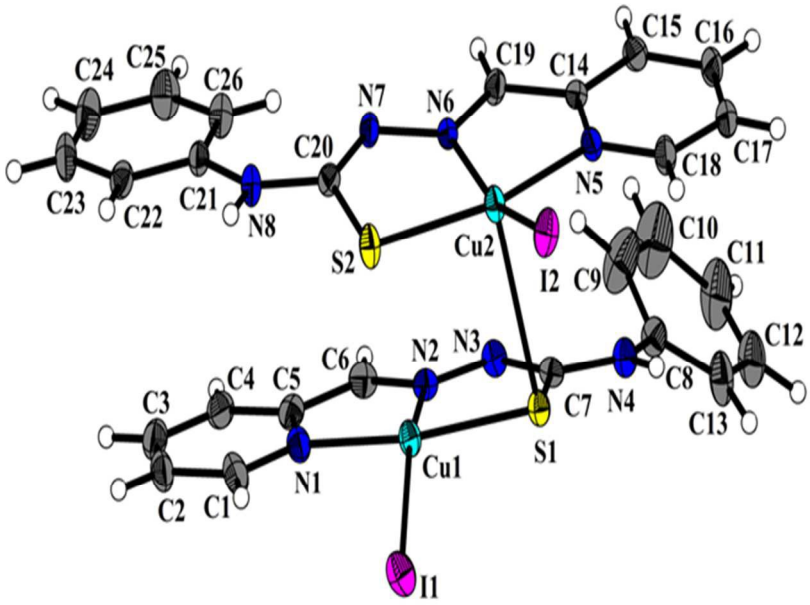


Fig. 3. ORTEP diagram of complex $[Cu_2^{II}I_2(\kappa^3:N,N,S\text{-}L^1\text{-}Ph)(\kappa^4:N,N,\mu\text{-}S\text{-}L^1\text{-}Ph)]$ **3** with 30% ellipsoid probabilty. Hydrogen atoms are omitted for more structure clarity. Complex **6** has similar structure (See SI, Fig S1)

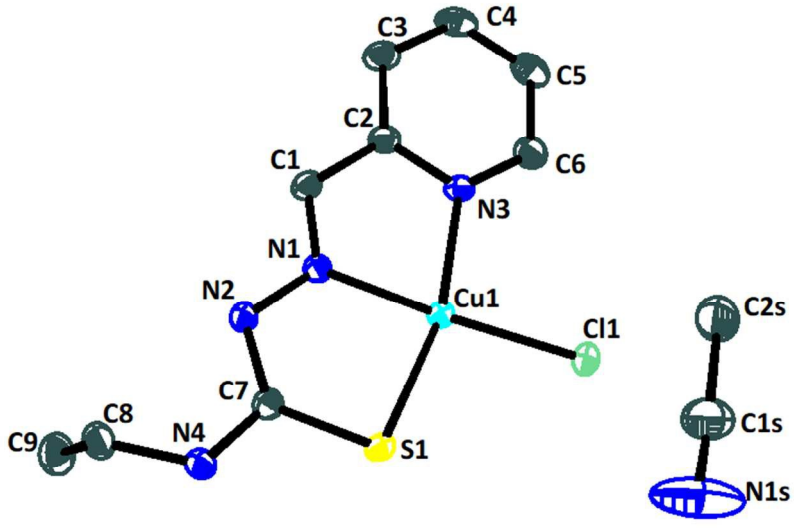


Fig. 4. ORTEP diagram of complex $[Cu^{II}Cl(\kappa^3:N,N,S\text{-}L^1\text{-}Et)]$ **8** with 30% ellipsoid probabilty. Hydrogen atoms are omitted for more structure clarity.

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The C-$N_{(hydrazinic)}$ and C-$N_{(amino)}$ bond distances fall in the ranges, 1.309 to 1.330 Å and 1.332 to 1.364 Å respectively. The bond lengths of the C-N and C-N(amino) bonds fall in the range typical for C=N double bonds.[58] Similarly, C-S bond distances fall in the range, 1.743 to 1.763 Å which is in between the single C-S bond distance of 1.81 and C-S double bond distance of 1.69 Å.[58] This again suggests partial double bond character of C-S bonds in complexes. Thus there is deprotonation of N-$H_{(hydrazinic)}$ moiety followed by delocalization of charge density between, C-S and C-$N_{(hydrazinic/amino)}$ bonds.

### 3.3 ESR Spectroscopy

The X-band ESR spectra of microcrystalline samples of complexes **1-9** were recorded to support divalent state of copper(II). The ESR parameters (g, A and G -exchange parameter) along with Figures S2-S10 are placed in Supporting Information and here a few observations are presented. Due to the distortion in geometry and the dipolar broadening induced by the closest neighbors in the crystal lattice, coupling from $^{63}Cu$ (I = 3/2) nucleus in the parallel and the perpendicular regions is not observed. Complexes **1** and **8** have shown partial $^{63}Cu$ coupling signals in the II regions and the ESR spectra of **1**, **4** and **8** conform to axial symmetry with the g parameters in the trend : $g_{II} > g_{\perp} > 2$ which implies that $^2B_1$ is the ground state of copper(II) in these complexes.[60-62] Complexes **3**, **6** and **7** have shown rhombic ESR spectra with $g_1$, $g_2$ and $g_3$ values falling in the range, 2.027 to 2.372, while complexes **2**, **5** and **9** have shown exchange broadened isotropic spectra with the $g_{iso}$ values in the range, 2.072 to 2.084.

### 3.4 ESI-Mass Studies

ESI-mass studies of complexes **1**, **2**, **4**, **7** and **9** have shown molecular ions $[Cu(\kappa^3:N,N,S\text{-}L^1\text{-}Me)I+2H]^+$ (**1**), $[Cu(\kappa^3:N,N,S\text{-}L^1\text{-}Et)I\text{-}H]^+$(**2**), $[Cu(\kappa^3:N,N,S\text{-}L^1\text{-}Me)Br\text{-}H]^+$(**4**), $[Cu(\kappa^3:N,N,S\text{-}L^1\text{-}Me)Cl + H]^+$(**7**) and $[Cu(\kappa^3:N,N,S\text{-}L^1\text{-}Ph)Cl]^+$ (**9**) (see **Figures** 5-9 for molecular ion spectra with isotopic pattern) (**A** - type species; Chart 4) {M represents complex, $[Cu(\kappa^3: N,N,S\text{-}L^1\text{-}R)X]$}. Complexes **3**, **5** and **6** have shown only **B** type species, $[M\text{-}X]^+$, with the loss of a halogen atom (Chart 4). Finally, complex **8** have shown the formation of both **B**-type $[M\text{-}X]^+$ and **C**-type species, $[M_2X]^+$, while complexes **7** and **9** have formed all three types of species (**A**, **B**, **C**). The

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formation of species **C** might have occurred when coordinated sulfur of species **A** acted as a bridge to form a bond to Cu of species **B** (see Supporting Information for more details of ESI-mass data, Figs S11-S19).

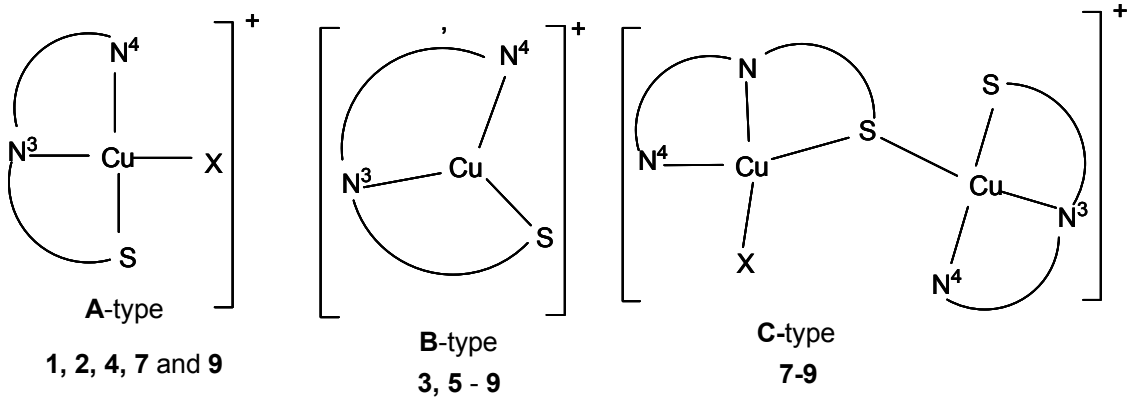


**Chart 4**

Chart 4. Main species identified using ESI-mass studies

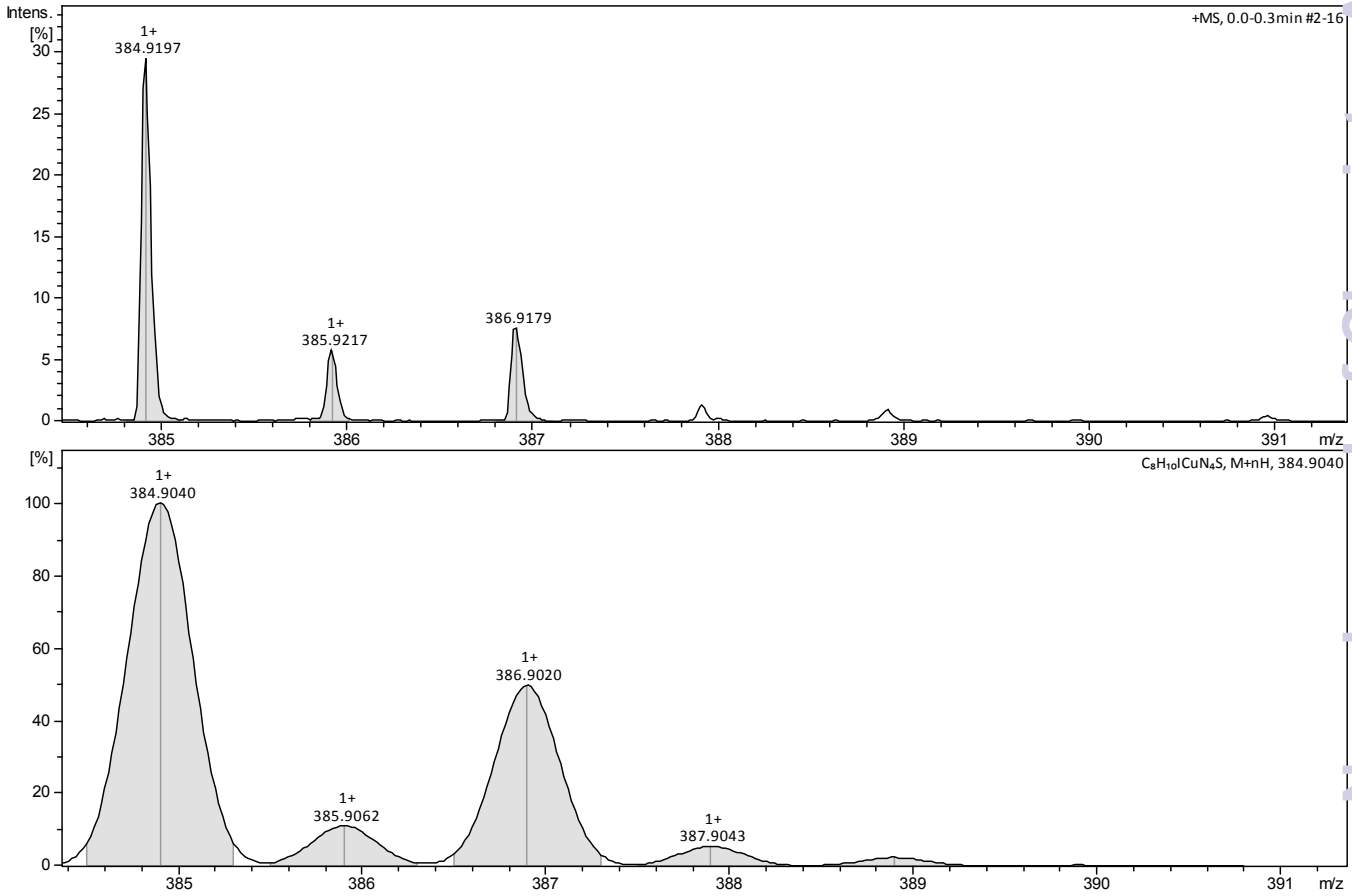

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Fig 5. ESI-mass spectrum of $[Cu(\kappa^3:N,N,S\text{-}L^1\text{-}Me)I+2H]^+$ (chemical formula: $CuC_8H_{10}N_4SI$; m/z = calcd 384.90, obsd 384.92) with isotropic pattern (Complex **1** ).

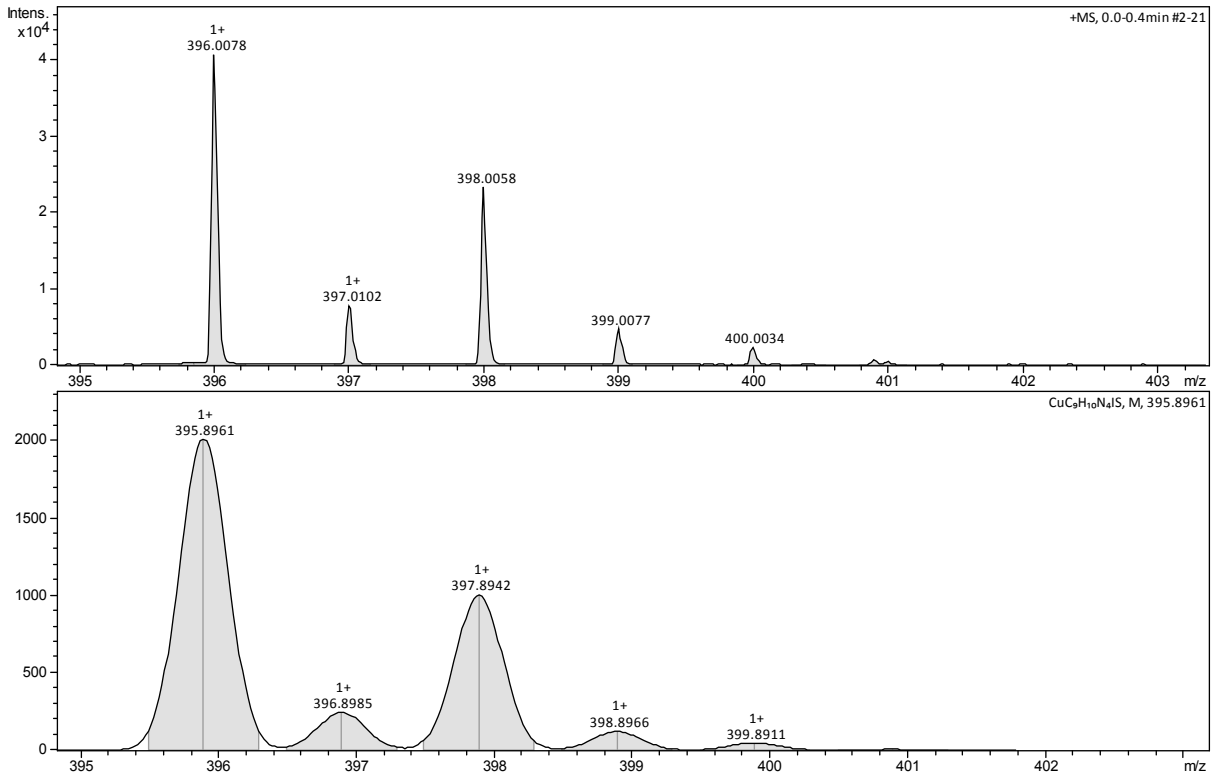


Fig. 6. ESI-mass spectrum of $[Cu(\kappa^3:N,N,S\text{-}L^1\text{-}Et)I\text{-}H]^+$ (chemical formula: $CuC_9H_{10}N_4IS$; m/z = calc 395.89, obsd 396.01) with isotropic pattern (complex **2** )

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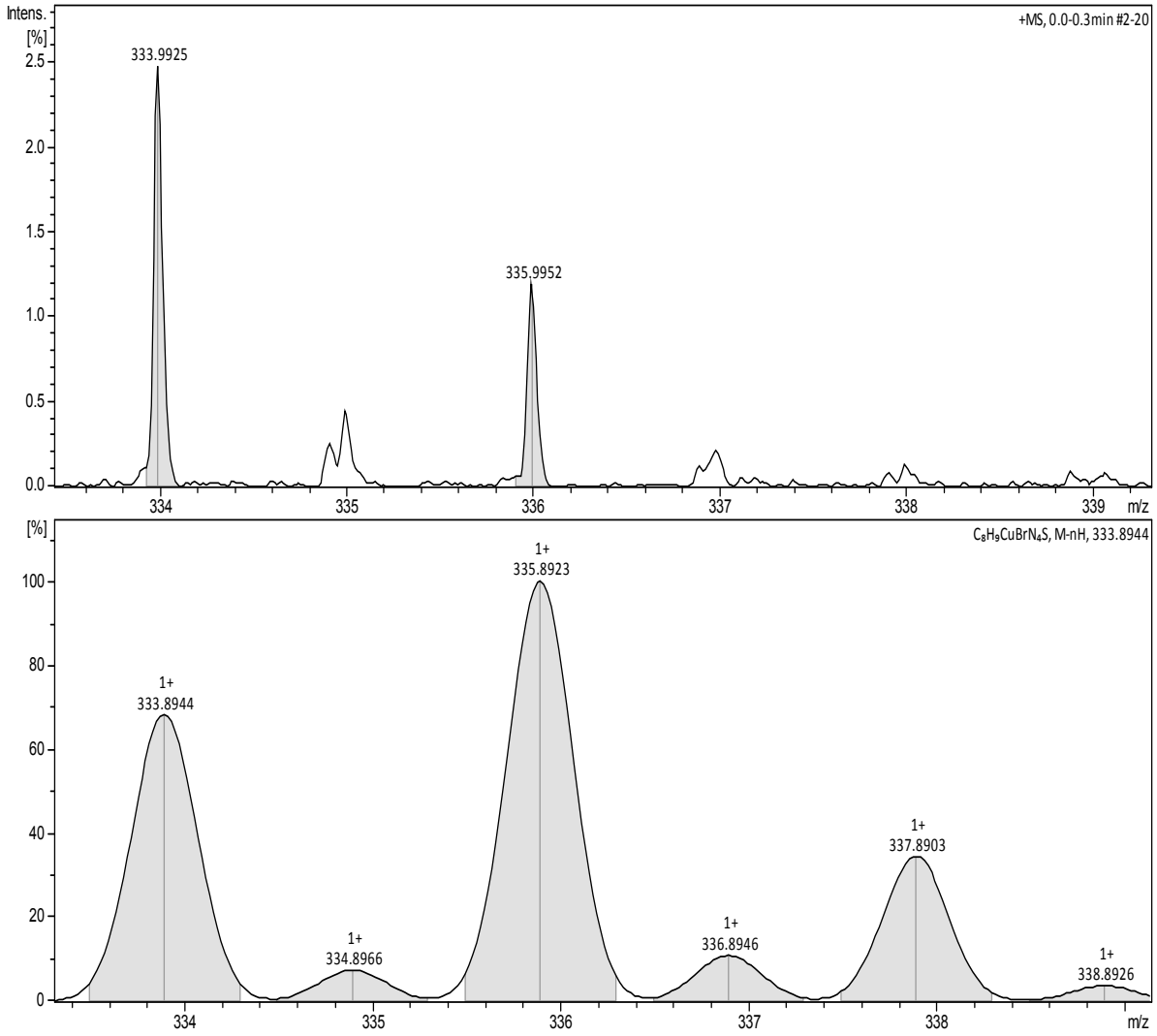


Fig. 7. ESI-mass spectrum of molecular ion $[Cu(\kappa^3:N,N,S\text{-}L^1\text{-}Me)Br\text{-}H]^+$ (chemical formula: $CuC_8H_8N_4SBr$; m/z = calcd 333.89, obsd 333.99) with isotropic pattern(complex **4** )

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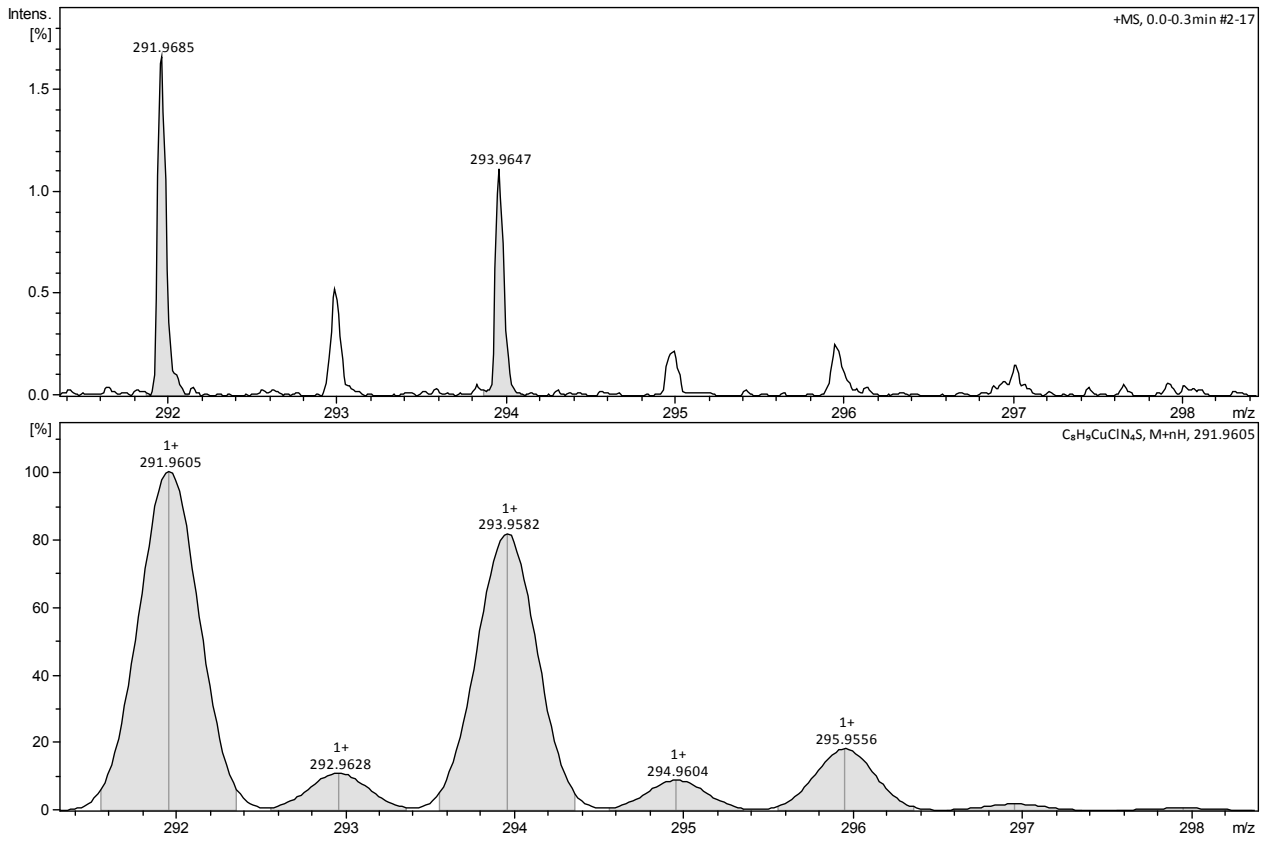


Fig. 8. ESI-mass spectrum of $[Cu(\kappa^3\text{:N,N,S-L}^1\text{-Me})Cl + H]^+$ (chemical formula: $CuC_8H_{10}N_4SCl$ ; m/z = calcd 291.96, obsd 291.97) with isotropic pattern (complex 7)

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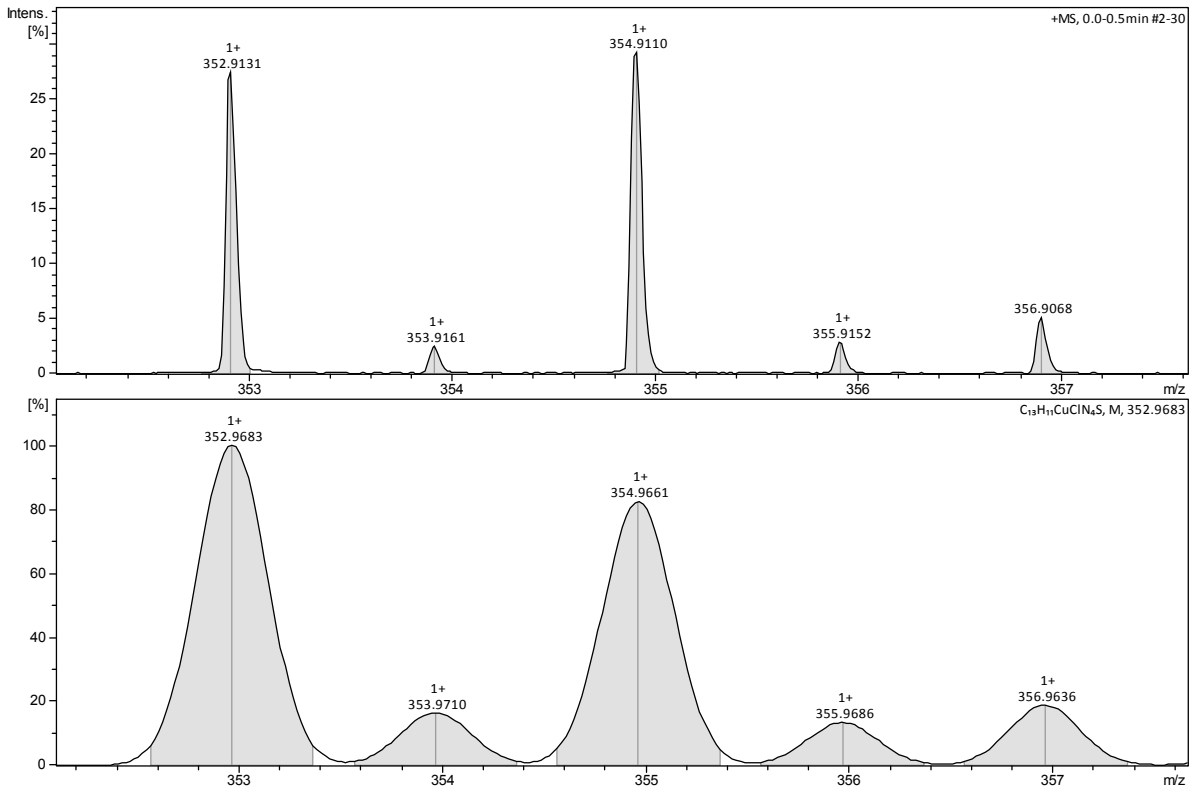


Fig. 9. ESI-mass spectrum of $[Cu(\kappa^3\text{:N,N,S-L}^1\text{-Ph})Cl]^+$ (chemical formula: $CuC_{13}H_{11}N_4SCl$; m/z = calcd 352.97, obsd 352.91) with isotropic pattern (complex **9** )

*3.5. Electronic Absorption spectroscopy*

In order to obtain electronic absorption bands in the UV region, $10^{-4}$ M solutions of complexes in DMSO were used and for observing d-d transitions, $10^{-3}$ M solutions of complexes were used. The electronic spectral bands of $10^{-3}$ M solutions occurred in the region, 615–680 nm ($\epsilon = 0.3 \times 10^2 - 1.73 \times 10^2$ L mol$^{-1}$cm$^{-1}$) and are probably due to d-d transitions involving energy levels: $^2B_1(d_{x2-y2}) \leftarrow {}^2E_g(d_{xz},d_{yz})$, which are characteristic of the copper compounds having a divalent oxidation state of the metal. At $10^{-4}$ M concentration, the bands observed in the region 274-307nm ($\epsilon = 0.79 \times 10^4 - 3.55 \times 10^4$ L mol$^{-1}$cm$^{-1}$) are attributed to the **π →π*** transitions and at 333-358nm ($0.123 \times 10^4 - 2.78 \times 10^4$ Lmol$^{-1}$cm$^{-1}$) are due to **n→π*** transitions. The spectral bands at the region 401-417nm ($0.653 \times 10^4 - 3.085 \times 10^4$ L mol$^{-1}$cm$^{-1}$) are attributed to the S → $Cu^{II}$ LMCT transitions (See Figs. S22-S27 given in supporting information for electronic absorption spectra of complexes).[45-47] It was found that UV-visible spectra of complexes remain unchanged in DMSO under variable conditions such as recording of the spectra after different

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intervals of time and after stirring a complex for a long period of time. The identical spectra were obtained in acetonitrile solution. It supports that DMSO acts as a solvent and complexes remain unchanged in this solvent {see Figures S20 – S27, supporting information}. It may be noted that crystal growth of complex **8** from DMSO-acetonitrile-methanol (1:1:1, v/v) did not support any rupture of Cu-X bond or replacement of thio-ligand by DMSO.

### 3.5 Antimicrobial activity and biosafety of the compounds

It is highlighted here that the studied microorganisms cause a variety of infections and become resistant to various types of antibiotics and thus pose a continuous challenge to the researcher to look for alternative antimicrobial agents. For example, methicillin resistant *Staphylococcus aureus* (MRSA) is a resistant form of *Staphylococcus aureus*. This paper was aimed to prepare new complexes of pyridine-2-formaldehyde thiosemicarbazones and to develop new metallo-organic antimicrobial agents based on N, S-donor thio-ligands,[45- 49] which could have potential antimicrobial activity. In order to justify their possible commercialization their biosafety has been worked out by MTT assay. Copper based metallo-organic compounds, namely, new complexes **1-9** with the thio-ligands ($HL^1$-R) as well as the previously reported[44] analogous complexes with the thio-ligands ($HL^2$-R) (see Chart 2 for ligands), namely, $[Cu_2^{II}I_2(\kappa^4:N,N,\mu\text{-}S\text{-}L^2\text{-}Me)_2]$ (**10**), $[Cu^{II}I(\kappa^3:N,N,S\text{-}L^2\text{-}Et)]$ (**11**), $[Cu^{II}I(\kappa^3:N,N,S\text{-}L^2\text{-}Ph)]$ (**12**), $[Cu^{II}Br(\kappa^3:N,N,S\text{-}L^2\text{-}Me)]$ (**13**), $[Cu^{II}Br(\kappa^3:N,N,S\text{-}L^2\text{-}Et)]$ (**14**), $[Cu^{II}Br(\kappa^3:N,N,S\text{-}L^2\text{-}Ph)]$ (**15**), $[Cu^{II}Cl(\kappa^3:N,N,S\text{-}L^2\text{-}Me)]$ (**16**), $[Cu^{II}Cl(\kappa^3:N,N,S\text{-}L^2\text{-}Et)]$ (**17**) and $[Cu^{II}Cl(\kappa^3:N,N,S\text{-}L^2\text{-}Ph)]$ (**18**), were tested against various bacteria, namely, methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*(MTCC740), *Klebsiella pneumonia* (MTCC530), *Shigella flexneri*(MTCC1457), *Salmonella typhimurium*1 (MTCC98), *Salmonella typhimurium*2 (MTCC1251), *Escherichia coli* (MTCC119), *Staphylococcus epidermidis* (MTCC435) and one yeast, *Candida albicans* (MTCC227). The results are delineated as under.

#### 3.5.1 The antimicrobial activity and minimum inhibitory concentration (MIC)

The antimicrobial activity of the compounds has been tested in terms of zone of inhibition by Agar well Diffusion Assay (ADA) followed by their Minimum Inhibitory Concentration (MIC) and Time Kill Assay. All complexes tested have shown antimicrobial activity against the clinical isolate methicillin resistant *Staphylococcus aureus* (MRSA), gram positive bacteria *viz.*

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*Staphylococcus aureus* (MTCC740), *Staphylococcus epidermidis* (MTCC435); gram negative bacteria, namely, *Klebsiella pneumoniae* (MTCC530), *Shigella flexneri* (MTCC1457), *Salmonella typhimurium*1 (MTCC98), *Salmonella typhimurium* 2 (MTCC1251), *Escherichia coli* (MTCC119), and yeast, *Candida albicans* (MTCC227). Most of the tested complexes showed potential antimicrobial activity in terms of zone of inhibition (ZOI) ranging from, 12 to 37 mm as well in terms of spectrum of activity (Table 2). Relative to metal complexes, the thio-ligands / metal halides showed either less or no antimicrobial activity (Tables 2 and 3).

Complexes of both types of thio-ligands {**1-9**, $HL^1$-R; **10-18,** $HL^2$-R} are active against methicillin resistant *Staphylococcus aureus* (MRSA) bacteria with zone of inhibition (ZOI) in the range 27-37 mm. Complexes **2, 4, 5, 8, 12** and **15** showed activity comparable or higher (ZOI, 33-37 mm) than that of the gentamicin (ZOI, 33 mm) (Table 2). The MIC values of active complexes fall in the range, 1 to 7 µg/mL, the lowest MIC of 1 µg/mL was observed in the case of complexes **2, 4, 5, 8, 9, 12, 13, 15** and **16,** which is much less as compared to that of standard gentamicin showing an MIC of 10 µg/mL (Table 3). *Staphylococcus aureus* (MTCC740) was sensitive to several complexes with an ZOI of 24-30 mm, which is comparable to or higher than that of the gentamicin (ZOI, 26 mm). Here complexes **2**, **5, 6**, **8**, **11** and **18** have the lowest MIC of 5-7 µg/mL, which is close to MIC of gentamicin (0.5 µg/mL). Other complexes have MIC of 10-500 µg/mL.

Among gram positive bacteria, *Staphylococcus epidermidis* (MTCC435) was the most sensitive organism (ZOI, 18-33 mm) towards different complexes. Complexes **2**, **4-6, 8-9, 11-18** showed better activity (ZOI, 27-33 mm) as compared to that of gentamicin (ZOI, 25 mm). Several complexes active against gram positive *Staphylococcus epidermidis* (MTCC435) have an MIC of 1-7 µg/mL which is less than that of the reference gentamicin (MIC = 30 µg/mL). The activity of these complexes against *Salmonella typhimurium* 2 (MTCC1251) was in the range, 26-37 mm (ZOI), with three complexes, namely, **2, 5** and **8** displaying higher antimicrobial activity with an ZOI of 37 mm, which is slightly better to that of the standard gentamicin 35.5 mm, on the other hand complexes **1, 4, 10, 12, 13** and **16** showed a comparable activity with ZOI of 31-34 mm. Most of complexes showed the lowest MIC of 1 µg/mL, which is comparable to that of the reference gentamicin (MIC = 1 µg/mL). Complexes **10**, **11**, **13**, **14**, **16** and **18** showed activity against *Candida albicans* (MTCC227) with ZOI values from 32-37 mm which is

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close to that of the reference amphotericin (ZOI of 34 mm). Several complexes have the MIC values of 1 µg/mL each, which is comparable to that of the reference amphotericin (MIC = 0.1 µg/mL). Only complexes **2**, **4**, **5**, **8**, **12** and **15** were active against *Escherichia* coli (MTCC119) with an ZOI in the range, 22-29 mm, which is close to that of the reference compound (ZOI, 30.5 mm); other complexes were either inactive or showed low activity. Among the active complexes, **12** and **15** have an MIC of 5 µg/mL which is equal to that of the standard gentamicin (Table 3).

*Salmonella typhimurium*1 (MTCC98) was sensitive to four complexes (**2**, **4**, **5**, **8**) with an ZOI of 23 - 28 mm, less than that of the reference gentamicin (ZOI = 40 mm) and MIC is high relative to reference (MIC, 1 µg/mL). Likewas the antimicrobial activity against *Shigella flexneri* (MTCC1457) was shown only by three complexes **2**, **4** and **5** with an ZOI of 19-22 mm which are much less than that of the gentamicin (ZOI = 34.5 mm; MIC, 5 µg/mL) and MIC was also high, 50 µg/mL. *Klebsiella pneumoniae* (MTCC530) was sensitive to several complexes (ZOI, 25-32 mm) and among these, three complexes **9**, **10**, **13** and **16** showed activity with an ZOI of 30 - 32 mm which is comparable to that of the standard gentamicin (ZOI = 33 mm). These complexes have an MIC of 1-5 µg/mL which is close to that of the reference compound (MIC = 0.5 µg/mL).

**Table 2 Biological data of complexes 1-18**[a,b,c]

| Complex/ ligand | C. a. 227[e] | MRSA[f] | K.p. 530[g] | S.a. 740[h] | *S.t.*2 1251[i] | *S.e.* 435[j] | E.c. 119[k] | *S.t.*1 98[l] | S.f. 1457[m] |
|---|---|---|---|---|---|---|---|---|---|
| Pyridine-2-carbaldehyde-N-substituted thiosemicarbazonates of copper(II), [CuX(N,N,S-$L^1$-R)] (R = Me, Et, Ph) | | | | | | | | | |
| **1**$_{(I, Me)}$ | 26 | 30 | 14 | 21 | 31 | 25 | 20 | 18 | NA |
| **2**$_{(I, Et)}$ | 29 | 37 | 25 | 28 | 37 | 30 | 25 | 28 | 22 |
| **3**$_{(I, Ph)}$ | 29 | 29 | 27 | 24 | 29 | 25 | NA | NA | NA |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **4**$_{(Br, Me)}$ | 26 | 35 | 20 | 25 | 34 | 28 | 24 | 23 | 19 |
| **5**$_{(Br, Et)}$ | 29 | 37 | 25 | 28 | 37 | 30 | 25 | 28 | 22 |
| **6**$_{(Br, Ph)}$ | 30 | 30 | 26 | 27 | 30 | 27 | NA | NA | NA |
| **7**$_{(Cl, Me)}$ | 22 | 27 | 28 | 20 | 27 | 21 | NA | NA | 13 |
| **8**$_{(Cl, Et)}$ | 27 | 35 | 20 | 27 | 37 | 28 | 22 | 24 | 16 |
| **9**$_{(Cl, Ph)}$ | 28 | 30 | 29 | 24 | 28 | 27 | NA | NA | NA |
| 2-Benzoylpyridine-N-substituted thiosemicarbazonates of copper(II), | | | | | | | | | |
| [CuX(N,N,S-L$^2$-R)] (R = Me, Et, Ph) | | | | | | | | | |
| **10**$_{(I, Me)}$ | 35 | 30 | 30 | 29 | 32 | 18 | 15 | 15 | 13 |
| **11**$_{(I, Et)}$ | 33 | 27 | 27 | 23 | 26 | 28 | NA | NA | NA |
| **12**$_{(I, Ph)}$ | 30 | 34 | 28 | 28 | 32 | 30 | 29 | NA | NA |
| **13**$_{(Br, Me)}$ | 35 | 32 | 30 | 28 | 31 | 28 | 16 | 14 | 15 |
| **14**$_{(Br, Et)}$ | 32 | 27 | 28 | 24 | 27 | 33 | NA | NA | NA |
| **15**$_{(Br, Ph)}$ | 30 | 33 | 29 | 26 | 30 | 30 | 28 | NA | NA |
| **16**$_{(Cl, Me)}$ | 37 | 31 | 32 | 23 | 32 | 29 | 16 | 16 | 14 |
| **17**$_{(Cl, Et)}$ | 31 | 29 | 27 | 24 | 29 | 31 | NA | NA | NA |
| **18**$_{(Cl, Ph)}$ | 33 | 28 | 28 | 23 | 28 | 30 | NA | NA | NA |
| Thio-ligands, HL$^1$-R and HL$^2$-R (R = Me, Et, Ph | | | | | | | | | |
| HL$^1$-Me | 14 | 17 | NA | NA | 18 | NA | NA | NA | NA |
| HL$^1$-Et | 16 | 23 | NA | 15 | 25 | 14 | NA | NA | NA |
| HL$^1$-Ph | 13 | 15 | NA | 14 | 16 | 14 | NA | NA | NA |
| HL$^2$-Me | 20 | NA | 14 | NA | 18 | NA | NA | NA | NA |
| HL$^2$-Et | 20 | 20 | 25 | NA | NA | NA | 21 | NA | NA |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $HL^2$-Ph | 16 | 19 | 16 | 20 | NA | NA | 21 | NA | NA |
| CuCl | 14 | 16 | 14 | 14 | 26 | NA | 13 | NA | NA |
| CuBr | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| CuI | 21 | 20 | 15 | 18 | 26 | 16 | 21 | 14 | 14 |
| Amphotericin[d] | <…… | | | | | | | | Gentamicin[d] |
| | …… | | | | | | | | |
| | 34 | 33[d] | 33 | 26 | 33.5 | 25 | 30.5 | 40 | 34.5 |

[a]All measurements are in mm diameter of the inhibition zone (N.A. indicates no activity). [b] The standard deviation varied in the range 0-1 based on three readings. [c] Studies were made in dmso. [d]Commercially available anti-microbial agents. [e]*Candida albicans* (MTCC227), [f]Methicillin resistant *Staphylococcus aureus,* [g]*Klebsiella pneumonia* (MTCC530), [h]*Staphylococcus aureus* (MTCC740), [i]*Salmonella typhimurium*2(MTCC1251), [j]*Staphylococcus epidermidis (*MTCC435), [k]*Escherichia coli* (MTCC119), [l]*Salmonella typhimurium* 1 (MTCC98),.[m]*Shigella flexneri* (MTCC1457), Gentamicin acts as positive control against bacteria {*MRSA, K. pneumoniae, S. aureus, S. typhimurium1, S. typhimurium2, S. epidermidis, E. coli, S. typhimurium2, S. flexneri*) and amphotericin acts as a positive control against yeast (*C. albicans*).

**Table 3** Minimum inhibitory concentration of $Cu^{II}$ complexes **1–18**[a] and thio-ligands

| Complex/ ligand | C. a. 227 | MRSA | K.p. 530 | S.a. 740 | *S.t.*2 1251 | *S.e.* 435 | E.c. 119 | *S.t.*1 98 | S.f. 1457 |
|---|---|---|---|---|---|---|---|---|---|
| **1**$_{(I, Me)}$ | 5 | 5 | 750 | 50 | 1 | 50 | 50 | 500 | 750 |
| **2**$_{(I, Et)}$ | 1 | 1 | 50 | 5 | 1 | 5 | 50 | 50 | 50 |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **3**$_{(I, Ph)}$ | 5 | 5 | 10 | 50 | 1 | 5 | ND | ND | ND |
| **4**$_{(Br, Me)}$ | 5 | 1 | 50 | 50 | 1 | 5 | 50 | 50 | 500 |
| **5**$_{(Br, Et)}$ | 1 | 1 | 50 | 5 | 1 | 5 | 50 | 50 | 50 |
| **6**$_{(Br, Ph)}$ | 1 | 5 | 10 | 5 | 1 | 5 | ND | ND | ND |
| **7**$_{(Cl, Me)}$ | 50 | 5 | 50 | 500 | 5 | 50 | 750 | ND | ND |
| **8**$_{(Cl, Et)}$ | 5 | 1 | 50 | 5 | 1 | 5 | 50 | 50 | 500 |
| **9**$_{(Cl, Ph)}$ | 5 | 1 | 5 | 50 | 5 | 5 | ND | ND | ND |
| **10**$_{(I, Me)}$ | 1 | 5 | 1 | 10 | 5 | 500 | 500 | 500 | 500 |
| **11**$_{(I, Et)}$ | 1 | 5 | 5 | 5 | 7 | 7 | ND | ND | ND |
| **12**$_{(I, Ph)}$ | 1 | 1 | 5 | 50 | 1 | 1 | 5 | ND | ND |
| **13**$_{(Br, Me)}$ | 1 | 1 | 1 | 50 | 1 | 50 | 500 | 500 | 500 |
| **14**$_{(Br, Et)}$ | 1 | 7 | 5 | 10 | 1 | 1 | ND | ND | ND |
| **15**$_{(Br, Ph)}$ | 1 | 1 | 5 | 50 | 1 | 1 | 5 | ND | ND |
| **16**$_{(Cl, Me)}$ | 1 | 1 | 1 | 50 | 1 | 5 | 500 | 500 | 500 |
| **17**$_{(Cl, Et)}$ | 1 | 7 | 5 | 50 | 7 | 1 | ND | ND | ND |
| **18**$_{(Cl, Ph)}$ | 1 | 5 | 5 | 7 | 5 | 1 | ND | ND | ND |
| $HL^1$-Me | 500 | 750 | ND | 750 | 50 | 750 | ND | ND | ND |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $HL^1$-Et | 500 | 50 | ND | 500 | 10 | 500 | ND | ND | ND |
| $HL^1$-Ph | 500 | 750 | ND | 500 | 750 | 500 | ND | ND | ND |
| $HL^2$-Me | 50 | ND | 750 | ND | 500 | ND | ND | ND | ND |
| $HL^2$-Et | 50 | 50 | 10 | 750 | ND | ND | 50 | ND | ND |
| $HL^2$-Ph | 500 | 50 | 500 | 50 | ND | ND | 50 | ND | ND |
| CuCl | 500 | 500 | 750 | 750 | 10 | ND | 750 | ND | ND |
| CuI | 50 | 50 | 750 | 500 | 10 | 500 | 50 | 500 | 500 |
| Amphotericin | <…… | | | | | | | | Gentamicin |
| | …… | | | | | | | | |
| | 0.1 | 10 | 0.5 | 0.5 | 1 | 30 | 5 | 1 | 5 |

[a]MIC in μg $mL^{-1}$

### 3.5.2 Time kill assay

The microbicidal or microbistatic nature of a antimicrobial compound can be ascertained by time kill assay. Two bacteria, namely, methicillin resistant *Staphylococcus aureus* (MRSA), *Salmonella typhimurium* 2 (MTCC1251) and one yeast reference strain, *Candida albicans* (MTCC227) were investigated as representative examples keeping in view high antimicrobial efficacy and corresponding lower MIC values. Fig. 10 shows the time kill assay graphs (percent killing versus time) of selected complexes **2-4, 9, 11, 18** (see SI for time kill assay graphs Fig.28-31 of other complexes tested **5**, **8**, **13**, **16**). From Figs. 11 and 12, it is seen that complete killing of MRSA occurred during 2-8 hours by different complexes. The lowest time is taken by complexes **4**, **5** and **8**, while complexes **9** and **16** took relatively more time for complete killing of the test organisms**.** Complete killing of *Salmonella typhimurium* 2 (MTCC1251) occurred in 2-8 h, the fastest killing was shown by complex **5.** With respect to yeast *C. albicans*

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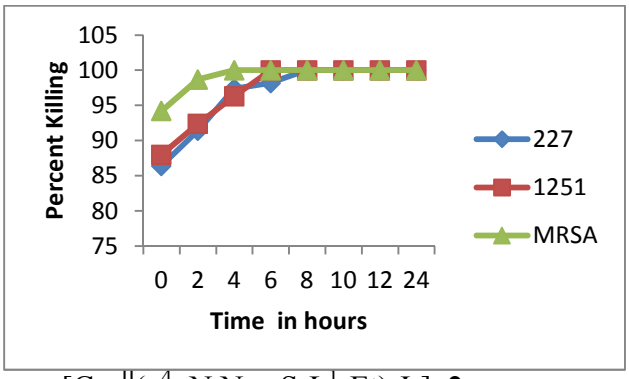
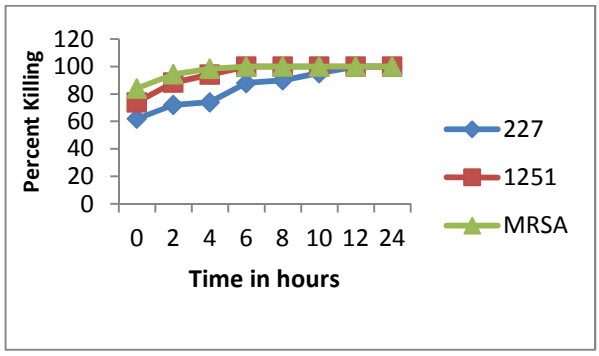
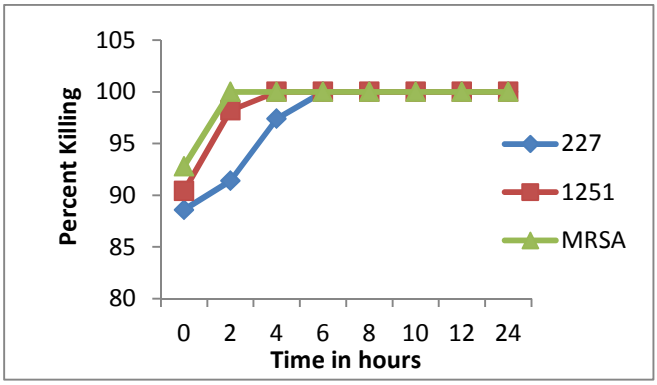
(MTCC227), the total time taken for complete killing was 2-12 h, the lowest time was taken by complex **11** followed by complex **5**.



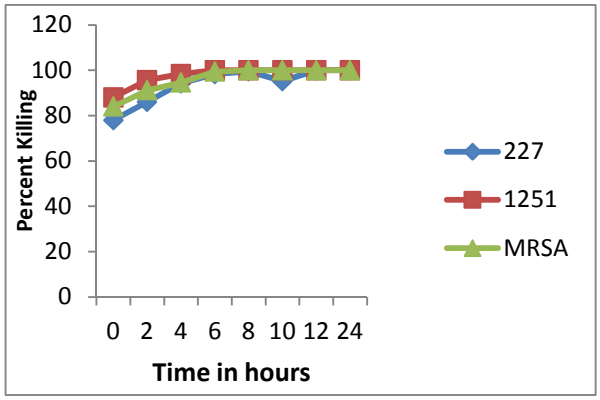

a. $[Cu_2^{II}(\kappa^4: N,N,\mu\text{-}S\text{-}L^1\text{-}Et)_2I_2]$ **2**



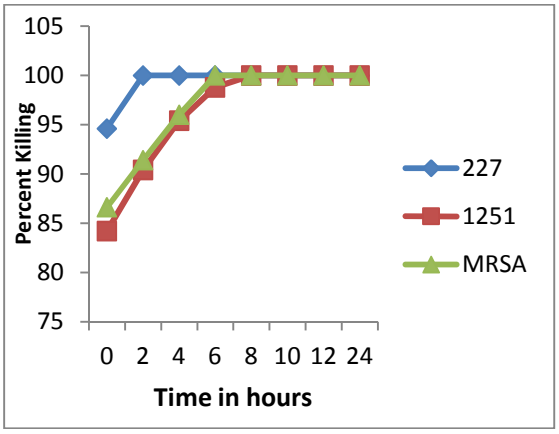

b.$[Cu_2^{II}(\kappa^3:N,N,S\text{-}L^1\text{-}Ph)(\kappa^4:N,N,\mu\text{-}S\text{-}L^1\text{-}Ph)I_2]$ **3**

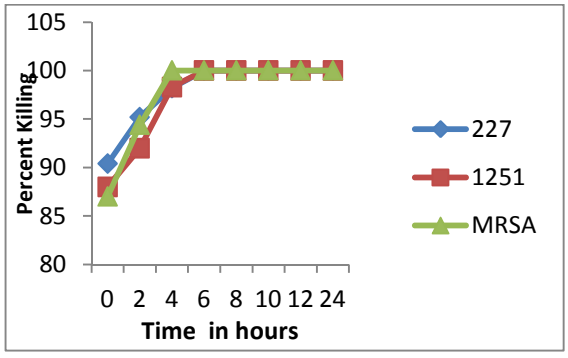



c. Complex $[Cu^{II}(\kappa^3:N,N,S\text{-}L^1\text{-}Me)Br]$ **4**




d. $[Cu^{II}(\kappa^3:N,N,S\text{-}L^1\text{-}Ph)Cl]$ **9**



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e. $[Cu^{II}I(\kappa^3\text{-N,N,S-}L^2\text{-Et})]$ **11** f. $[Cu^{II}Cl(\kappa^3\text{-N,N,S-}L^2\text{-Ph})]$ **18**

Fig. 10. Time kill assay graphs of complexes **2-4**, **9**, **11** and **18** as a function of time (see SI for time kill assay graphs of other complexes tested **5**, **8**, **13** and **16**).

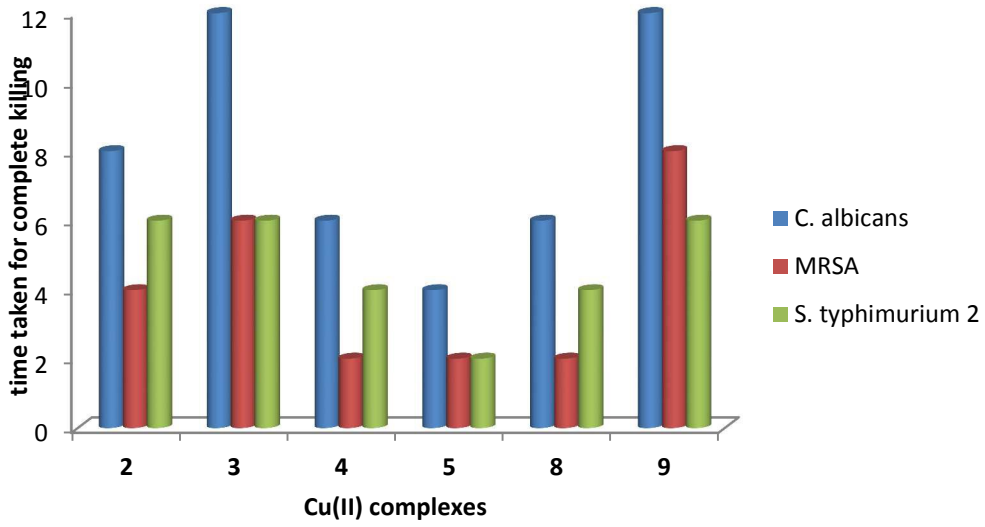


Fig. 11. Time taken for complete killing of C.albicans, MRSA and S. typhimurium 2 by the complexes **2-5**, **8** and **9**)

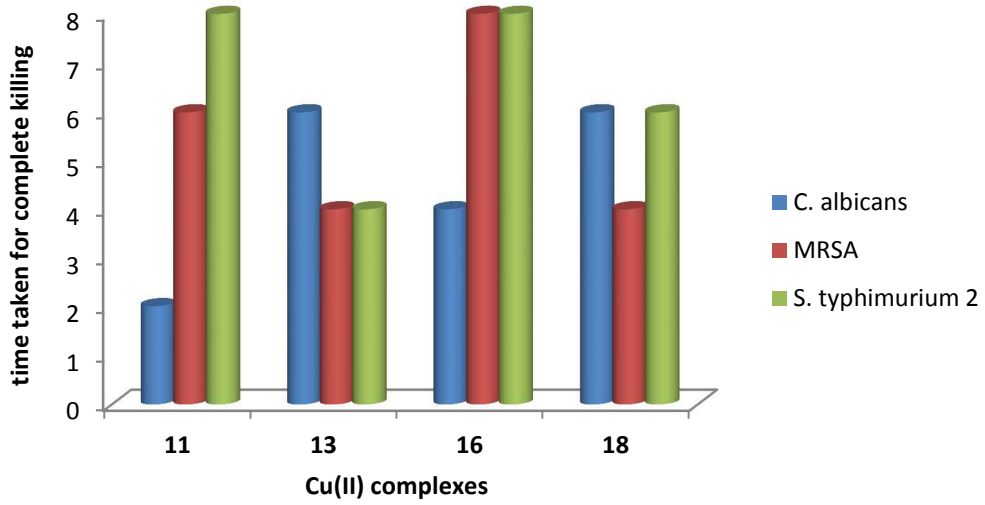

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Fig 12. Time taken for complete killing of C.albicans, MRSA and S. typhimurium 2 by the complexes **11**, **13**, **16** and **18**

### 3.5.3 Cellular toxicity testing using MTT assay

Complexes **1-18** have been tested for cellular toxicity using the MTT cytotoxicity assay which is measured colorimetrically {MTT = 3-[(4,5-dimethylthiazol-2-yl)-2,5-diphenyl] tetrazolium bromide}.[63] This measurement of cytotoxicity assay is based on the capacity of mitochondrial succinate dehydrogenase enzymes in sheep blood cells to reduce the yellow water soluble substrate MTT into an insoluble purple colored formazan product which is measured spectrophotometrically. The reduction of MTT occurs only in metabolically active cells, wherein MTT is converted into insoluble formazan crystals which are dissolved by dimethyl sulfoxide and the absorbance of reaction mixture corresponds to cellular viability. Complexes when treated with sheep blood cells showed comparable cellular viability to that of control (Table 4). Cellular viability for seventeen complexes varied in the range of 90-98%; complex **6** showed lowest value of 84.3%. Highest cell viability (95-98%) is shown by complexes **2**, **10**, **13**, **15** and **17**. Complexes of 2-benzoylpyridine-N-substituted thiosemicarbazones showed higher cell viability over 2-pyridineacrabaldehyde-N-substituted thiosemicarbazones.

Table 4 The cell viability in presence of complexes

| Complex | Optical density | % cell viability | Complex | Optical density | % cell viability |
|---|---|---|---|---|---|
| $1_{(I, Me)}$[a] | 0.916 | 93.2 | $10_{(I, Me)}$[b] | 0.852 | 96.2 |
| $2_{(I, Et)}$[a] | 0.939 | 95.5 | $11_{(I, Et)}$[c] | 0.876 | 94.9 |
| $3_{(I, Ph)}$[b] | 0.827 | 93.3 | $12_{(I, Ph)}$[b] | 0.818 | 92.3 |

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| | | | | | |
|---|---|---|---|---|---|
| $4_{(Br, Me)}$[a] | 0.908 | 92.4 | $13_{(Br, Me)}$[b] | 0.862 | 97.3 |
| $5_{(Br, Et)}$[a] | 0.925 | 94.1 | $14_{(Br, Et)}$[c] | 0.871 | 94.4 |
| $6_{(Br, Ph)}$[b] | 0.747 | 84.3 | $15_{(Br, Ph)}$[c] | 0.887 | 96.1 |
| $7_{(Cl, Me)}$[b] | 0.820 | 92.6 | $16_{(Cl, Me)}$[b] | 0.804 | 90.7 |
| $8_{(Cl, Et)}$[a] | 0.917 | 93.3 | $17_{(Cl, Et)}$[c] | 0.889 | 96.3 |
| $9_{(Cl, Ph)}$[b] | 0.836 | 94.3 | $18_{(Cl, Ph)}$[c] | 0.849 | 92.0 |

Control (DMSO) : optical density = 0.983[a]; 0.886[b]; 0.923[c]. % Cell viability = (OD of treated complex / OD of control) x 100

---

### 3.5.4. A comparison of antimicrobial activity with literature reports

Here it may be appropriate to compare the antimicrobial activity of complexes against bacterial strains reported in literature. In literature, the related thio-ligand, 3-acetylpyridine-N-(2-pyridyl)thiosemicarbazone (D Type)[30] formed complex, $[Cu(HL)_2Cl_2]$, which showed antimicrobial activity (ZOI, 16 mm) against *E. Coli* somewhat more than that of reference compound, ampicillin (ZOI, 14 mm). This copper complex, $[Cu(HL)_2Cl_2]$, also showed comparable or higher activity against P. *aeruginosa,* S. *pasteuri*, B. *thuringiensis* , E. *carotovora* and *yeast* than the reference compound.[30] Among the complexes under study, **2**, **4**, **5**, **8**, **12** and **15** are active against *Escherichia* coli (MTCC119), and among these complexes **12** and **15** have ZOI values of 28-29 mm, close to that of the reference compound, gentamicin (ZOI, 30.5 mm) and have MIC of 5 μg/mL, which is equal to that of the standard (MIC, 5 μg/mL).

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The activity of complexes **2**, **5** and **8** (ZOI, 37 mm) against *Salmonella typhimurium* 2 (MTCC1251) (ZOI, 37 mm) is slightly better to that of the standard gentamicin, ZOI = 35.5 mm. These complexes have the lowest MIC of 1 μg/mL, which is comparable to that of the reference gentamicin (MIC = 1 μg/mL). *Salmonella typhimurium* 1 (MTCC98) was sensitive to only four complexes (**2**, **4**, **5**, **8**) with an ZOI of 23 - 28 mm and MIC of 50 μg/mL for each complex, and these values are less than that of the gentamicin (ZOI = 40 mm; MIC = 1 μg/mL). In literature, copper(II) chlorides formed complexes with pyridine-2-formaldehyde-$N^1$-tolyl thiosemicarbazones (Chart1, A type Ligand, $R_1$ = H; $N^1HR$ , $HL^3$), pyridine-2-carbaldehyde-$N^1$-tolyl thiosemiacrabazones (Chart1, A type Ligand, $R_1$ = Me; $N^1HR$, $HL^4$) and 2-benzoylpyridine-$N^1$-tolyl thiosemiacrabazones { Chart 1, A type Ligand: $R_1$ = Ph, $N^1HR$, $HL^5$), having stoichiometry, $[Cu(HL^3)Cl_2]$ (R = o-tolyl, m-tolyl, p-tolyl), $[Cu(HL^4)Cl]$ (R = o-tolyl, m-tolyl, p-tolyl); $[Cu(HL^5)Cl_2]$ (R = o-tolyl, m-tolyl, p-tolyl).[31,32] These complexes have shown antimicrobial activity against Salmonella typhimurium (ATCC13311) with MIC in the range, 2.40 - 11.04 μg $mL^{-1}$ which are higher than those shown by several complexes under study against *Salmonella typhimurium* 2 (MTCC1251). There is no ZOI data given in the paper for comparison.[31]

All complexes showed activity against *Candida albicans* (MTCC227) where the ZOI ranged from 26-37 mm, the highest activity was shown by the complexes, **10**, **11**, **13**, **14**, **16** and **18** (ZOI, 32-37 mm) which is close to or higher than that of the reference compound, amphotericin (ZOI of 34 mm). Twelve complexes have MIC of 1 μg/mL which is a bit higher to that of the reference amphotericin (MIC = 0.1 μg$mL^{-1}$). In literature,[31,32] complexes $[Cu(HL^3)Cl_2]$, $[Cu(HL^4)Cl]$ and $[Cu(HL^5)Cl_2]$ (Chart 1, A type ligands, $R_1$ = H, $HL^3$; Me, $HL^4$;

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Ph, $HL^5$; $N^1HR$; R = o-tolyl, m-tolyl, p-tolyl)[31,32] have shown MIC in the range, 0.14- 2.67 $\mu gmL^{-1}$, but no ZOI data is given in paper.[31,32]

Copper(II) complexes of 2-benzoylpyridine-$N^1$-phenylthiosemicarbazone ($HL^2$-Ph; Chart 2) with composition, [Cu($L^2$-Ph)X] (X = Cl, Br, $NO_3$, NCS, $N_3$][33] were found active against *vibrio cholerae* O1,[33] with ZOI, 9-10 mm at MIC of 5 μg $mL^{-1}$ for each complex. Only complex, [Cu($L^2$-Ph)$N_3$] was active against *Salmonella paratyphi* with ZOI of 9 mm at MIC of 4 μg $mL^{-1}$.[33] None of the complexes was active against *Staphylococcus aureus* except the ligand itself which showed activity with ZOI of 12 mm at MIC of 4 μg $mL^{-1}$.[33] In contrast, nearly all complexes (**1-18**) tested by our group are sensitive to *Staphylococcus aureus* (MTCC740) and among them, complexes **2**, **5, 6**, **8**, **11** and **18** have ZOI of 23-28 mm, comparable to that of gentamicin (ZOI, 26 mm), but these complexes have MIC values of 5-7 μg/mL, which are higher than that of gentamicin (0.5 μg/mL).

All the complexes tested are active against methicillin resistant *Staphylococcus aureus* (MRSA) bacteria and among them, complexes **2, 4, 5, 8, 12** and **15** showed activity comparable or higher (ZOI, 33-37 mm) than that of the reference compound gentamicin with ZOI of 33 mm and MIC of 1 μg/mL, while standard gentamicin showed an MIC of 10 μg/mL. It is added here that there is no report of metal complexes of pyridine based thiosemicarbazones with which comparison can be made.[30-33] *Staphylococcus epidermidis* (MTCC435) was highly sensitive organism (ZOI, 25-33 mm) towards different complexes. Except **1**, **7**, **10** and **13**, other complexes with ZOI of 25-33 mm have an MIC of 1-7 μg/mL which is much less than that of the reference gentamicin (MIC = 30 μg/mL). *Shigella flexneri* (MTCC1457) sensitive to only three complexes (**2**, **4**, **5**) with an ZOI of 19-22 mm less than that of the gentamicin (ZOI = 34.5 mm) and have high MIC of 50 μg/mL relative to gentamicin (MIC, 5 μg/mL). Complexes **9**, **10**, **13**

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and **16** showed activity *Klebsiella pneumoniae* (MTCC530) with an ZOI of 30 - 32 mm which is comparable to that of the standard gentamicin (ZOI = 33 mm), but MIC of 1-5 μg/mL is close to that of the reference compound (MIC = 0.5 μg/mL).

## 4 Conclusions

A novel series of copper(II) complexes with central coordination core : [CuX($N^4$,$N^3$,S-$L^{1,2}$-R] (X = I, Br, Cl; R = Me, Et, Ph) involving coordination through pyridiyl nitrogen- $N^4$, azomethine nitrogen-$N^3$ and thiolato sulfur, possess stable $Cu^{II}$-iodine, $Cu^{II}$-bromine, and $Cu^{II}$-chlorine bonds. The formation of these compounds eventually occurred by the reactions of copper(I) halides (CuX, X = I, Br, Cl) with pyridine-2-carbaldehdye- and 2-benzoylpyridne-N-substituted thiosemicarbazones via a proton coupled electron transfer mechanism as reported earlier.[43,44] The current investigations describe antimicrobial activity, MIC, time kill assay and MTT studies not reported for any set of complexes in literature. Further, complexes tested showed high antimicrobial potential against different microbial strains, namely, MRSA, *Staphylococcus aureus* (MTCC740), *Staphylococcus epidermidis* (MTCC435), *Klebsiella pneumoniae* (MTCC530) etc. and were found to be relatively biosafe as evident from high cell viability (90-98%) obtained using MTT cytotoxicity assay. This study provides a background for future biochemical investigations and may emerge as i*n vivo biosafe* antimicrobial agents.

## Conflicts of interest

There are no conflicts to declare.

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Graphical abstract

Copper(II) complexes with pyridine based thiosemicarbazones, have shown high antimicrobial potential against different microbial strains, and were found to be biosafe with several complexes displaying high cell viability (90-98%) obtained using MTT cytotoxicity assay.