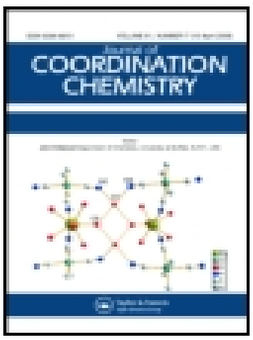

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Synthesis, Antimicrobial Activity and High Cell Viability of Copper Derivatives of 2-Thiouracil, Purine-6-thione and 2,4,6-Trimercaptotriazine

Jaspreet Kaur Aulakh,[a] Samridhi Sharma,[a] Henna Sood,[b] Daljit S. Arora,[b] Manpreet Kaur,[c] Jerry P. Jasinski[c] and Tarlok S. Lobana,[a*]

[a]Department of Chemistry, Guru Nanak Dev University, Amritsar – 143 005, India;

[b]Department of Microbiology, Guru Nanak Dev University, Amritsar – 143 005, India;

[c]Department of Chemistry, Keene State College, Keene NH 03435–2001 USA

*Corresponding ng author : Tarlok S. Lobana; e-mail address: **tarlokslobana @yahoo.co.in** ; **tslasr@gmail.com**

A B S T R A C T

Reactions of copper(II) acetate with 2- thiouracil ($tucH_2$) and triphenyl phosphine (1 : 1 : 1 or 1 : 1 : 2 molar ratios) in methanol-acetonitrile / chloroform mixture yielded a light brown N,S-bridged dinuclear Cu^I complex, $[Cu(PPh_3)_2(\mu\text{-}N,S\text{-}tucH)Cu(PPh_3)_2Cl]$ **1**, incorporating uninegative 2-thiouracilate. X-ray crystallography has shown that the crystals of complex **1** comprise a two component twin system and belong to the space group **Ia.** The major component **1a** is about 77% and the minor component **1b** is about 23%. One Cu is four-coordinate tetrahedral with center $\{CuP_2SCl\}$ and the other Cu is three-coordinate distorted

trigonal planar with {CuP_2N} center. Crystal data: **1**, formula, $C_{76}H_{63}ClCu_2N_2OP_4S$; space group : Ia; monoclinic, a, 22.2638(9) ; b, 11.8050(4) ; c, 27.4455(10) Å ; β, 113.358(5)◦; R, 4.80%;173(2) K. The antimicrobial activities of dinuclear **1** as well as that of the previously reported 2-thiouracil, 2,4,6-trimercaptotriazine ($tmtH_3$) and purine-6-thione ($purSH_2$) complexes, [$CuCl(\kappa^1$-S-$tucH_2)(PPh_3)_2$] **2**, [$Cu_2Br_2(\mu$-S-$tucH_2)_2(PPh_3)_2$] **3,** [$Cu(tmtH_2)(PPh_3)_2$] **4**, [$Cu_3Br_2(k^1$-N,k^1-S,μ-S-$tmtH_2)(PPh_3)_6$] **5** and [$Cu(k^1$-N,k^1-S-purSH)$(PPh_3)_2$] **6**, have been screened against gram negative, *Escherichia coli* (MTCC 119) and *Shigella flexneri* (MTCC 1457), gram positive, *Enterococcus faecalis* (MTCC 439) and *Staphylococcus aureus* (MTCC 740) microorganism. High percentage of cell viability (96-97 %) is observed in some cases

KEYWORDS

2-Thiouracil; Copper(II); 2,4,6-trimercaptotriazine; purine-6-thione; Triphenyl phosphine

1. Introduction

Heterocyclic-2- thiones bearing functional moieties such as –N(H)-C(=S)-, -N(H)-C(=S)-N=, -N(H)-C(=S)-N(H)-, have several coordination modes with metals giving a variety of compounds of variable nuclearities [1-15]. Another interesting feature of this class of thio-ligands pertains to the chemical reactivity involving C-S rupture with copper halides forming an interesting class of compounds [16-21]. The coordination chemistry of thio-ligands, pyridine-2-thiones, pyrimidine- 2-thiones, 1,3-imidazolidine-2-thiones and their derivatives has been the main focus of several researchers [1-15], while other multifunctional thio-ligands such as 2-thiouracil, 2,4-dithiouracil, purine-6-thione and 2,4,6-trimercaptotriazine have found much less attention [22-31].

We have been interested in coordination chemistry with different metals and chemical reactivity of heterocyclic-2-thiones involving C-S bond rupture under ambient conditions [15-23, 29, 30] and more recently the applications of metal complexes (Cu, Ag) with imidazolidine-2-thiones as antimicrobial agents have been reported [20, 32, 33]. In this paper, syntheses and antimicrobial activities of copper based complexes with multidentate thio-ligands as shown in Chart 1 are examined. A brief summary of the biological applications

of metal 2-thiouracil ($tucH_2$), 2,4,6-trimercaptotriazine (H_3tmt) and purine-6-thione ($purSH_2$) reported previously is presented here. For example, Au(I) and Pt(IV) complexes of 2-thiouracil are known for their anti-arthritic activities [34] and cytotoxic activities towards *A549* and *A2780* cell lines [35]. The 5-substituted-2-thiouracil derivatives have been used as anti-cancer [36] and anti-tumor drugs [37]. These derivatives inhibited the folate mechanism of cancer cells and were also useful as coronary vasodilators [37]. Similarly, Zn^{2+}, Fe^{2+} and Mn^{2+} complexes of H_3tmt have shown *in vitro* antitumor activities against *G-361* (human malignant melanoma), *HOS* (human osteogenic sarcoma), *K–562* (human chronic myelogenous leukemia) and *MCF-7* (human breast adenocarcinoma) tumor cell lines, along with antimicrobial activities against bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* [38]. Platinum(II) complexes of purine-6-thione have shown anticancer activity [39].

Thio-ligands, 2-thiouracil and 2,4-dithiouracil have limited coordination chemistry [22-31]. For example, 2-thiouracil with copper(II) chloride formed a mononuclear trigonal planar Cu^{I} complex, $[CuCl(\kappa^1\text{-S-}tucH_2)_2]$ [28], involving reduction of Cu^{II} to Cu^{I}. Reactions of copper(I) halides with $tucH_2$ and triphenylphosphine (PPh_3) yielded a tetrahedral complex, $[CuCl(\kappa^1\text{-S-}tucH_2)(PPh_3)_2]$ **2** [29] and a sulfur-bridged dinuclear complex, $[Cu_2Br_2(\mu\text{-S-}tucH_2)_2(PPh_3)_2]$ **3**

[30]. The related thio-ligand, 2,4-dithiouracil with copper(I) halides formed a mononuclear [CuBr(κ^2-P,P-dppbz)(κ^1-S-dtucH$_2$)] {dppbz = 1,2-bis(diphenylphosphanyl)benzene}[31], dinuclear [Cu$_2$(μ-X)(κ^1-S,κ^1-S-dtucH)(PPh$_3$)$_4$] (X = Cl, Br) [29] and polynuclear [Cu(μ-S,S-dtucH$_2$)(PPh$_3$)X]$_n$ (X = Cl, Br, I) complexes [22]. Silver(I) halides with dtucH$_2$ yielded dinuclear complexes, [Ag$_2$(μ-X)(κ^1-S,κ^1-S-dtucH)(PPh$_3$)$_4$] (X = Cl, Br) [23]. The multidentate thio-ligands, tmtH$_3$ and purSH$_2$, with copper(I) formed complexes, [Cu(tmtH$_2$)(PPh$_3$)$_2$] **4** [29] , [Cu$_3$Br$_2$(k^1-N,k^1-S,μ-S-tmtH$_2$)(PPh$_3$)$_6$] **5** [29] and [Cu(k^1-N,k^1-S-purSH)(PPh$_3$)$_2$] **6** [29].

In this paper, synthesis of a dinuclear complex, [Cu(PPh$_3$)$_2$(μ-N,S-tucH)Cu(PPh$_3$)$_2$Cl] **1,** its antimicrobial activities, along with that of the previously reported **2-6** [29, 30] are reported. Complexes have been screened against gram negative, *Escherichia coli* (MTCC 119) and *Shigella flexneri* (MTCC 1457), gram positive *Enterococcus faecalis* (MTCC 439) and *Staphylococcus aureus* (MTCC 740) microorganism.

2. Experimental

2.1. Materials and techniques

2-Thiouracil, copper(II) acetate dihydrate and triphenyl phosphine were procured from Aldrich Sigma Ltd. Elemental analyses (CHNS) were carried out using the THERMO FINNIGAN FLASH technique. Infrared spectra were recorded using KBr pellets from 4000-200 cm^{-1} on a Pye–Unicam SP-3-300 spectrophotometer. Melting points were determined with an electrically heated Gallenkamp apparatus. ^{1}H NMR spectra were recorded on a Bruker

Avance II 400 NMR FT spectrometer operating at 400 MHz using DMSO-d_6 as the solvent with TMS as the internal standard. ESI-mass spectra were recorded in DMSO using a Bruker Daltonik LS-MS high resolution microTOF-Q II 10356.

2.2. Synthesis of [Cu(PPh$_3$)$_2$(μ-N,S-tucH)Cu(PPh$_3$)$_2$Cl] (1)

A solution of 2- thiouracil (0.016 g, 1 mmol) in methanol (15 mL) was added to a solution of copper(II) acetate (0.025 g, 1 mmol) in acetonitrile (8 mL). The contents were stirred at room temperature overnight and the black precipitate formed was separated. To this precipitate was added triphenylphosphine (0.060 g, 2 mmol) dissolved in chloroform (10 mL) and the contents were refluxed for one hour; addition of a few milliliters of methanol made the solution clear. This solution was left at room temperature for two weeks when light brown crystals were formed. Yield: 0.070 g, 66%, m. p., 190 -195 ^{0}C, C, H, N, S analysis for $C_{76}H_{63}ClCu_2N_2OP_4S$ (1338.73): C, 68.00; H, 4.70; N, 2.09; S, 2.39. Found: C, 67.83; H, 4.96; N, 2.14; S, 2.21. IR data (KBr, cm^{-1}) : 3176 (w, N-H); 3070 (m), 3051 (m), 2933 (w), 2882 (m), 2796(m) (C-H); 1648 (s, C=O), 1585 (s, C-N); 1532 (s), 1480s, 1434 w, 1388 w (νC-C + δC-H); 1329w, 1283s; 1157 (m, C-S); 1094 (s, P-C); 1027 w, 998 w; 922w, 848 w, 812 w, 744 s, 695 s, 618 w, 572 w, 452 w. ^{1}H NMR (DMSO-d_6, δ, ppm): 12.23 (sb, 1H, NH); 8.2(sb, H, H^5), 7.58 (m, 24H, o-H, PPh_3), 7.50 (m, p-H, 12H, PPh_3); 7.34 (m, m-H, 24H, PPh_3 + H^6, Htuc$^-$). **1** is soluble in solvents such as DMSO, dichloromethane and methanol.

2.3. Preparation of previously reported complexes. Complexes [CuCl(κ^1-S-tucH$_2$)(PPh_3)$_2$] (**2**), [Cu$_2$Br$_2$(μ-S-tucH$_2$)$_2$(PPh_3)$_2$] (**3**), [Cu(tmtH$_2$)(PPh_3)$_2$] (**4**), [Cu$_3$Br$_2$(k^1-N,k^1-S, μ-S-tmtH$_2$)(PPh_3)$_6$] (**5**) and [Cu(k^1-N,k^1-S-purSH)(PPh_3)$_2$] (**6**) were prepared as reported earlier [29,30] and their

stoichiometry is confirmed through analytical data and ^{1}H-NMR data given in supporting information (see supporting information, Figure S 6a-f).

2.4. 2-Thiouracil Ligand

IR data (KBr, cm^{-1}) : 3200m, 3135 m (N-H); 3086m, 3050m, 2931m (C-H); 1708 (s, C=O), 1567 (s, C-N); 1450s, 1423 s, 1394 s (νC-C + δC-H); 1176 (m, C-S); 1097 (m), 1003 m, 964 w, 912 m, 896 m, 834 m, 761m, 737 w, 710 w, 549 s, 472 w. **^{1}H NMR** (DMSO-d_6, δ, ppm) : 12.37(s, 2H, NH), 7.36 (d, J = 8 Hz; 1H, H^5), 5.78 (d, J = 8; 1H, H^6).

2.5. X-ray crystallography

The crystal structure of **1** was determined by mounting a single crystal of the complex on a glass fiber and data were measured with Rigaku-Oxford Diffraction equipped with graphite monochromated Mo-Kα (λ = 0.71073 Å) radiation at 173(2) K. The structure was solved by direct methods and refined using full-matrix least-squares based on F^2 using ShelXL-2014. In this structure, all non-hydrogen atoms were refined anisotropically and the hydrogens have been fixed geometrically [40].

2.6. Antimicrobial studies

Test organisms. The reference strains of bacteria and yeast were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Reference strains included Gram positive bacteria: *Staphylococcus aureus* (MTCC 740), *Enterococcus faecalis* (MTCC 439), Gram negative bacteria *Shigella flexneri* (MTCC 1457), *Escherichia coli* (MTCC 119). A loopful of broths with bacterial and yeast colonies were inoculated into 5 mL of their respective medium and incubated at 37 °C and 25 °C, respectively,

for 4 h. This was used as inoculum after adjusting the turbidity as per the McFarland turbidity standard. This turbidity is equivalent to approximately (1 to 2) $\times 10^8$ colony forming units per mL (CFU/mL). The inoculums thus prepared were used for antimicrobial screening. The experimental details for antimicrobial screening and a test to check the level of cellular toxicity of the test compounds, MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay is given in supporting information.

3. Results and Discussion

3.1. Synthesis, IR and NMR spectroscopy

In a reaction of copper(I) chloride with 2-thiouracil and triphenylphosphine in 1 : 1 : 2 molar ratio in acetonitrile-methanol mixture, the product obtained was a tetrahedral complex, $[CuCl(\kappa S^1\text{-}tucH_2)(PPh_3)_2]$, as reported earlier [29]. When this reaction was carried out with 2-thiouracil and PPh_3 in the same molar ratio in acetonitrile-methanol mixture containing a few mL of DMSO, the product is $[Cu_2(PPh_3)_4(tucH)Cl]$ (tucH is 2-thiouracilate anion) [41]. Reaction of copper(II) acetate with 2-thiouracil in acetonitrile-methanol mixture followed by reaction with triphenylphosphine (1: 1 : 2 molar ratio) in chloroform-methanol mixture yielded a product $[Cu(PPh_3)_2(\mu\text{-}N,S\text{-}tucH)Cu(PPh_3)_2Cl]$ **1,** as confirmed by the analytical data and X-ray crystallography. The formation of **1** involves abstraction of chlorine from chloroform. Chart 2 shows the structure of **1** as well as those of previously reported **2** [29], **3** [30], **4-6** [29], which form part of the antimicrobial study in the present investigation.

IR spectra of the uncoordinated $tucH_2$ and **1** have been recorded from 4000 – 400 cm^{-1} and the major bands are reported in the experimental section. The thio-ligand showed two medium intensity ν(N-H) bands at 3200, 3135 cm^{-1} in **1**, one weak ν(N-H) band at 3176 cm^{-1} was

observed, suggesting deprotonation of one (N-H) moiety probably the one close to C^4=O group which was confirmed by the x-ray crystal structure of **1**. The thio-ligand $tucH_2$ showed one strong ν(C=O) band at 1708 cm^{-1} which shifted to 1648 cm^{-1} probably as a result of coordination of N^3 to Cu. Complex **1** has shown a ν(C-S) of medium intensity at 1157 cm^{-1} which occurs in the low energy region relative to free $tucH_2$ [ν(C-S)), 1176 cm^{-1}] and it supports the formation of metal-sulfur bonds. The appearance of ν(P-C_{Ph}) at 1094 cm^{-1} (strong in intensity) confirmed the presence of coordinated PPh_3 in **1** (see supporting information for complete spectra, Figure S1 and Figure S2).

The ^{1}H NMR spectrum of uncoordinated $tucH_2$ showed a broad signal at δ = 12.37 ppm, which corresponded to the combined N^1-H and N^3-H protons and in **1** a broad signal at δ = 12.23 ppm corresponds to N1-H proton after deprotonation of N^3-H moiety. The uncoordinated $tucH_2$ ring protons showed NMR signals due to C^6H and C^5H protons at δ 7.36 and 5.78 ppm, respectively (see SI: Figure S3a, S3b). The C^6H ring proton underwent a downfield shift upon coordination to Cu(I) and gave a single broad signal at δ = 8.28 ppm, whereas the C^5H ring proton merges with the absorption due to m-H protons of PPh_3 ligands. The o-H, m-H and p-H protons of coordinated PPh_3 are multiplets at 7.34 -7.58 ppm.

3.2. Crystal structure of [Cu(PPh$_3$)$_2$(μ-N,S-tucH)Cu(PPh$_3$)$_2$Cl] 1

The X-ray crystal structure determination revealed that monoclinic crystals of **1** comprise a two component twin system and belong to the space group Ia. The major component is about 77% and the minor component is about 23%. The crystal data are placed in Table 1 and the bond parameters are placed in Table 2. $[Cu_2(PPh_3)_4(tucH)Cl]$ (tucH is 2-thiouracilate anion) with similar stoichiometry formed triclinic crystals in Pī space group and exists as a single component

[41], unlike the two component twin system of **1**. Figure 1 shows the molecular structure of major component (**1a**; 77%), Figure 2 shows molecular structure of minor component (**1b**; 23%) and finally Figure 3 shows the overlapping major and minor components of **1**. Here the detailed discussion of major and minor components is presented.

In the major component **1a** (Figure 1**)**, Cu1 is bonded to one N1 and P1 and P2 at bond distances of 1.928(5), 2.2335(14) and 2.2666(14) Å, respectively, with bond angles of N-Cu-P2, 112.81(16), N-Cu-P1, 122.78(16) and P-Cu-P, 122.91(5)^{o}. These bond parameters support a distorted trigonal planar geometry of Cu1. Cu2 is bonded to P3, P4, S1 and one Cl1 donor at bond distances of 2.2658(13), 2.2912(15); 2.368(2) and 2.4471(18) Å, respectively, with bond angles of 120.16(5) -102.03(6)^{o}. These bond parameters support a distorted tetrahedral geometry of Cu2 (Figure 1).

In the minor component **1b**, the $tucH^{-}$ anion changes orientation leading to change of donor atoms of the two metal centers (Figure 2). Here Cu1 is bonded to S1A, P1, P2 and Cl1A at bond distances of 2.431(8); 2.2335(14), 2.2666(14) and 2.597(7) Å, respectively, with bond angles of 97.87(14) – 122.91(5)^{o} and these bond angles support distorted tetrahedral geometry of Cu1; Cu2 is bonded to N1A, P3 and P4 at bond distances of 1.768(14), 2.2658(13) and 2.2912(15) Å, respectively, with bond angles of 108.7(6), 120.15(5) and 129.9(6)^{o}. These bond angles support a distorted trigonal planar geometry of Cu1. In the two components, **1a** and **1b**, the distortions of two tetrahedral geometries as well as two trigonal planar geometries are different.

The coordination pattern of $[Cu_2(PPh_3)_4(tucH)Cl]$ (tucH is 2-thiouracilate anion) [41] is similar to say **1a**, one Cu is four coordinate ($CuClP_2S$, tetrahedral) and the second Cu is three-coordinate (CuP_2N, trigonal planar). The interesting feature is formation of a twin system in the

present case and medium might have played a role. An analogous thio-ligand, namely, 2,4,6-trimercaptotriazine ($tmtH_3$) with $Cu(OAc)(PPh_3)_2$ has one coordination pattern ($CuNP_2$), similar the three-coordination shown by **1a** or **1b**. This $tmtH_3$ with copper(I) bromide and PPh_3 showed a four-coordination pattern ($CuSP_2Br$) similar to the one shown by **1a** or **1b [**29].

In formation of **1**, the 2-thiouracilate anion ($tucH^-$) binds to one Cu centre through its N donor near C=O and two PPh_3 ligands. The electronegative N and two P donors stabilize Cu^I of $CuNP_2$. Further, $tucH^-$ also binds to another Cu via its S donor, two PPh_3 ligands and one chloride. The origin of chloride is traced to $CHCl_3$ and is necessary for stabilization of Cu^I when bonded to one S and two P donors, i.e. the core $CuSP_2$ abstracts halogen from $CHCl_3$ to generate $CuSP_2Cl$ in **1**. The 2-thiouracil with PPh_3 resulted in stabilization of Cu^I and no complex in Cu^{II} oxidation state could be obtained. Direct reaction of copper(II) chloride with 2-thiouracil yields, a three coordinate Cu^I complex, $[CuCl(\kappa^1\text{-S-}tucH_2)_2]$ [28] in which 2-thiouracil is a neutral ligand.

3.3. ESI-mass studies

The ESI-mass spectrum of **1** shows the formation of two types of species, $[Cu_2(PPh_3)_3(\mu\text{-N,S-tuc})]^+$ (A- type) and $[Cu_2(PPh_3)_2(\mu\text{-N,S-tuc})]^+$ (B-type). Figures 4 and 5 depict ESI-mass spectral peaks of A-type and B-type species, respectively, which are consistent with their isotopic patterns. The formation of A-type species reveals loss of one halide, one PPh_3 and that of B-type involves loss of one halogen and two PPh_3 molecules from **1.**

3.4. Antimicrobial activity and biosafety evaluation

$[Cu_2(PPh_3)_4(\mu\text{-}N,S\text{-}tucH)Cl]$ **1**, $[CuCl(k^1S\text{-}tucH_2)(PPh_3)_2]$ **2**, $[Cu_2Br_2(\mu\text{-}S\text{-}tucH_2)_2(PPh_3)_2]$ **3**, $[Cu(tmtH_2)(PPh_3)_2]$ **4**, $[Cu_3Br_2(k^1\text{-}N,k^1\text{-}S,\mu\text{-}S\text{-}tmtH_2)(PPh_3)_6]$ **5** and $[Cu(k^1\text{-}N,k^1\text{-}S\text{-}purSH)(PPh_3)_2]$ **6** [complexes **2-6**; 29, 30] show activity against *Staphylococcus aureus* from 12-18 mm (ZOI), the highest activity is shown by **5**. H_3tmt has activity of 14 mm against *S. aureus*, unlike 2-thiouracil and purine-6-thione, which were inactive. However, the structurally uncharacterized Cu^{II} complex, $[Cu(tucH_2)(NH_3)_4]Cl_2$ showed activity against *S. aureus* with a ZOI of 14 mm [42], while another Cu^{II} complex, $[Cu(tucH\text{-}6\text{-}NH_2)_2(H_2O)]\cdot 2H_2O$ showed low activity [43]. Complexes **1-3** show higher activity (ZOI of 16-21 mm) against *Shigella flexneri* as compared to 2-thiouracil (ZOI of 15 mm). There is no report with which comparison could be made [42, 43]. Only **1** showed somewhat higher activity against *E. faecalis* with ZOI of 16 mm in comparison to 2-thiouracil which showed activity of 14 mm, but **2-6** and free ligands, H_3tmt and $purSH_2$ were inactive. There is no report with which comparison could be made [42, 43]. Only 2-thiouracil showed antimicrobial activity (ZOI, 16 mm) against *E. Coli* bacteria, while all other complexes tested were inactive. The structurally uncharacterized Cu^{II} complexes showed either comparable activity, $\{[Cu(tucH_2)(NH_3)_4]Cl_2$, ZOI of 14 mm$\}$ [42] or very poor activity $\{[Cu(tucH\text{-}6\text{-}NH_2)_2(H_2O)]\cdot 2H_2O$ [43] against *E. Coli* bacteria. Activity of complexes/2-thiouracil as described above was less than that of the standard Gentamicin (Table 3).

3.4.1. Cellular toxicity using MTT assay

All of the complexes **1-6,** which were tested for their antimicrobial activity against various microorganisms, were evaluated for cellular toxicity using MTT assay[44]. This cytotoxicity assay is based on the capacity of mitochondrial succinate dehydrogenase enzymes in blood cells

to reduce the yellow water soluble substrate MTT into an insoluble purple formazan product which is measured spectrophotometrically. $[Cu(tmtH_2)(PPh_3)_2]$ (**4**) and $[Cu_3Br_2(k^1\text{-}N,k^1\text{-}S,\mu\text{-}S\text{-}tmtH_2)(PPh_3)_6]$ (**5**), showed purple color with optical density (OD) of 0.651 **(4)** and 0.642 **(5)**, respectively, at 590 nm wavelength. The OD of the control (untreated cells in DMSO) was 0.672. Therefore, these compounds were non-cytotoxic with 97% (**4**) and 96% (**5**) cell viability.

4. Conclusion

2-Thiouracil with copper(II) acetate yielded a dinuclear complex $[Cu_2(PPh_3)_4(\mu\text{-}N,S\text{-}tucH)Cl]$ **1** involving abstraction of chlorine from methanol-chloroform during re-crystallization; **1** has unsymmetrically bridged 2-thiouracilate ligand. The crystals of this complex are a two component twin system and belong to the space group **Ia.** The major component **1a** is about 77% and the minor component **1b** is about 23%. One Cu is four-coordinate tetrahedral with $\{CuP_2SCl\}$ and the second Cu is three-coordinate $\{CuP_2N\}$ with distorted trigonal planar geometry. The antimicrobial activities of **1** as well as those of previously reported **2-6** [29, 30] reveal activity against *S. aureus, Shigella flexneri and E. Faecalis.* On examining **4** and **5** for their *in vitro* cellular toxicity using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltrazolium bromide] assay, showed high cell viability of 97 % (**4**) and 96 % (**5**).

Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix A. Supplementary data

Crystallographic data for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository numbers CCDC – 1838783 (**1**) (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk). IR and NMR data of ligand and complex **1**, ESI-MS data of complex **1**, experimental details for antimicrobial activity of 2-thiouracil and complexes**.**

References

[1] P. D. Akrivos, *Coord. Chem. Rev.*, **213**, 181 (2001).

[2] E. S. Raper, *Coord. Chem. Rev.*, **129**, 91 (1994).

[3] E. S. Raper, *Coord. Chem. Rev.*, **153**, 199 (1996).

[4] E. S. Raper, *Coord. Chem. Rev.*, **165**, 475 (1997).

[5] J. A. Garcia-Vazquez, J. Romero and A. Sousa, *Coord. Chem. Rev.*, **193**, 691 (1999,).

[6] M. Hong, Y. Zhao, W. Su, R. Cao, M. Fujita, X. Zhou and A. S. C. Chan, *Angew. Chem., Int. Ed.*, **39**, 2468 (2000).

[7] D. Li, W.-J. Shi and L. Hou, *Inorg. Chem.*, **44**, 3907 (2005).

[8] L. Han, B. Wu, Y. Xu, M. Wu, Y. Gong, B. Lou, B. Chen and M. Hong, *Inorg. Chim. Acta*, **358**, 2005 (2005).

[9] T. S. Lobana, R. Sultana, A. Castineiras and R. J. Butcher, *Inorg. Chim. Acta*, **362**, 5265 (2009) .

[10] J. J. M. Amoore, L. R. Hanton and M. D. Spicer, *Dalton Trans.*, 1056 (2003)..

[11] R. Peng, D. Li, T. Wu, X. P. Zhou and S. W. Ng, *Inorg. Chem*., **45,** 4035 (2006).

[12] T. Wu, B.-H. Yi and D. Li, Inorg. Chem., **44**, 4130 (2005).

[13] J. A. Garcia-Vazquez, J. Romero, A. S. Pedrares, M. L. Louro, A. Sousa and J. Zubieta, J. Chem. Soc., *Dalton Trans*., 559 (2000).

[14] G. A. Bowmaker, J. V. Hanna, B. W. Skelton and A. H. White, *Chem. Commun*., 2168 (2009).

[15] T. S. Lobana, R. Sharma, G. Hundal and R. J. Butcher, *Inorg. Chem*., **45**, 9402 (2006).

[16] T. S. Lobana, Razia Sultana, Geeta Hundal, R. J. Butcher, *Dalton Trans.* **39**, 7870 (2010).

[17] T.S. Lobana, Razia Sultana, R.J. Butcher, *Dalton Trans* **40**, 11382 (2011).

[18] T. S. Lobana, Razia Sultana, R.J. Butcher, A. Castineiras, T. Akitsu, F. J. Fernandez, M. C. Vega, *Eur. J. Inorg. Chem.* 5161 (2013).

[19] Razia Sultana, Tarlok S. Lobana and Alfonso Castineiras, RSC *Adv* **5,** 100579 (2015)

[20] J. K. Aulakh, **T. S. Lobana**, H. Sood, D. S. Arora , I. Garcia-Santos, G. Hundal, M. Kaur, V. A. Smolenski, and J. P. Jasinski , *Dalton Trans.*, **46,** 1324 (2017) ; ibid, *Dalton Trans*., **46**, 15138 (2017) .

[21] T. S. Lobana, P. J. Kaur, Geeta Hundal and R. J. Butcher, *Z. Anorg Allg. Chem.* **638,** 2340 (2012).

[22] R. Sultana, T.S. Lobana, R. Sharma, A. Castineiras, T. Akitsu, K. Yahagi, Y. Arikate, *Inorg. Chim. Acta*, **363**, 3432 (2010).

[23] T.S. Lobana, R. Sultana, R.J. Butcher, J.P. Jasinski, J.A. Golen, A. Castineiras, K. Propper, F.J. Fernandez, M.C. Vega, *J. Organomet. Chem.*, **745-746**, 460 (2013).

[24] M.S. Masoud, O.H.A. El-Hamid, Z.M. Zaki, *Transition Met. Chem.*,**19**, 21 (1994).

[25] M. Ruf, K. Weis, H. Vahrenkamp, *Inorg. Chem.*, **36,** 2130 (1997).

[26] P. Kamlakannan, D. Venkappayya, *Russ. J. Coord. Chem.*, **28**, 423 (2002).

[27] J.R. Lusty, H.S.O. Chan, J. Peeling , *Transiton Met. Chem.*, **8**, 343 (1983).

[28] G.W. Hunt, E.A.H. Griffith, E.L. Amma, *Inorg. Chem.*, **15**, 2993 (1976).

[29] T.S. Lobana, A.K. Sandhu, R.Sultana, R.J. Butcher, A. Castineiras, J.P. Jasinski, *RSC Adv.*, **4**, 30511 (2014).

[30] T.S. Lobana, A. Kaur, R. Sharma, M. Bala, A. K. Jassal, C.E. Duff, J.P. Jasinski, *J. Chem. Sci.*, **127**, 1859 (2015).

[31] P. Aslanidis, P.J. Cox, A. Kaltzoglou, A.C. Tsipis, *Eur. J. Inorg. Chem.*, 334 (2006).

[32] J. K. Aulakh, T. S. Lobana, H. Sood, D. S. Arora, V. A. Smolinski, C. E. Duff, J. P. Jasinski, *J. Inorg. Biochem*. **178,** 18 (2018).

[33] T. S. Lobana, J. K. Aulakh, H. Sood, D. S. Arora, I. Garcia-Santos, M. Kaur, C. E. Duff, J. P. Jasinski, *New J. Chem.,* 42, 9886 (2018).

[34] C.S.W. Harker, E.R.T. Tiekink, M.W. Whitehouse, *Inorg. Chim. Acta*, **181,** 23, **(**1991) .

[35] C. Vetter, G.N. Kaluderovic, R. Paschke, R. Kluge, J. Schmidt, D. Steinborn, *Inorg. Chim. Acta*, **363 ,** 2452 (2010).

[36] G.W. Anderson, I.F. Halverstadt, W.H. Miller, R.O. Roblin Jr., *J. Am. Chem. Soc.*, **67**, 2197 (4945).

[37] A.F. Eweas, Q.A. Abdallah, E I. Hassan, J. *Appl. Pharma. Sci.*, **4**,102 (2014).

[38] P. Kopel, K. Dolezal, L. Machala, V. Langer, *Polyhedron*, **26,** 1583 (2007) .

[39] M. Das, S.E. Livingstone, *Br. J. Cancer*, **38** , 325 (1978).

[40] G.M. Sheldrick, *Acta Cryst.*, **C71**, 3 (2015).

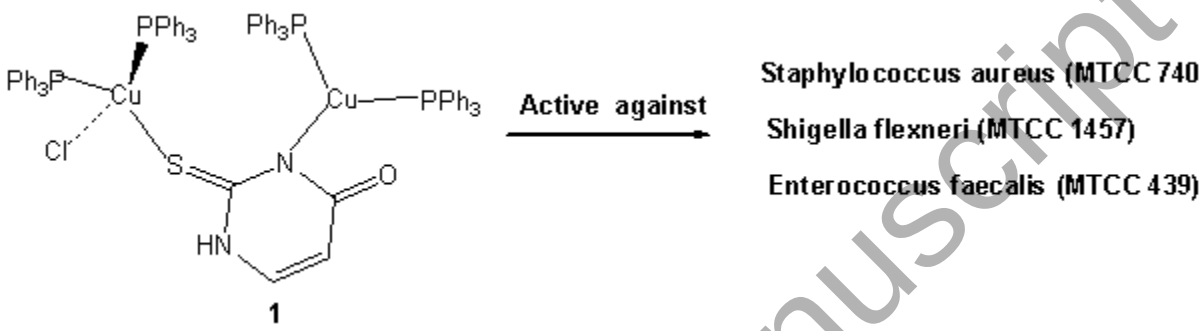
[41] D.C. Charalampou, N. Kourkoumelis, S. Karanestora, L.P. Hadjiarapoglou, V. Dokorou, S. Skoulika, A. Owczarzak, M. Kubicki, S.K. Hadjikakou, *Inorg. Chem.*, **53,** 8322 (2014).

[42] M.S. Masoud, A.A. Soayed, A.F. El-Husseiny, *Spectrochimica Acta Part A*, **99**, 365 (2012).

[43] M.S. Masoud, E.A. Khalil, A.M. Ramadan, Y.M. Gohar, A. Sweyllam, *Spectrochimica Acta Part A*, **67**, 669 (2007).

[44]J. G. Onsare and D. S. Arora, *J. Appl. Microbiol.*, **118**, 313 (2014).

Graphical abstract



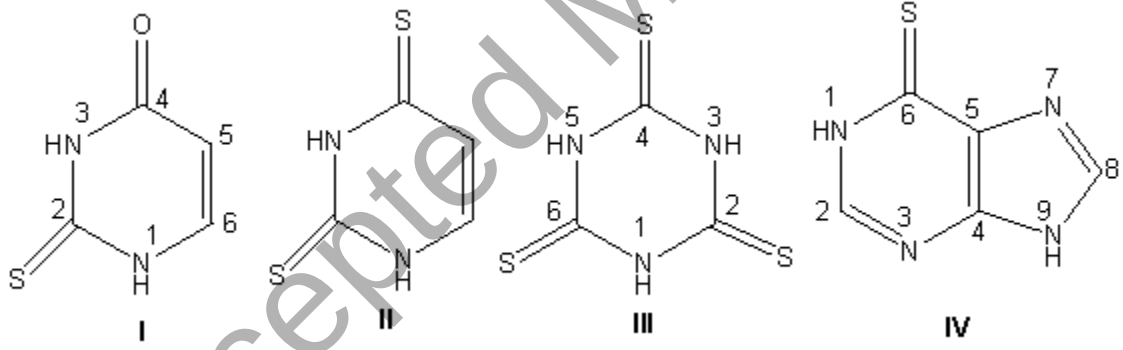


Chart 1. 1-Thiouracil and related thio-ligands: $tucH_2$ = 2-thiouracil (I); $dtucH_2$ = 2,4 – dithiouracil (II); $tmtH_3$ = 2,4,6-trimercaptotriazien (III); purSH2 = purine-6-thione (IV)

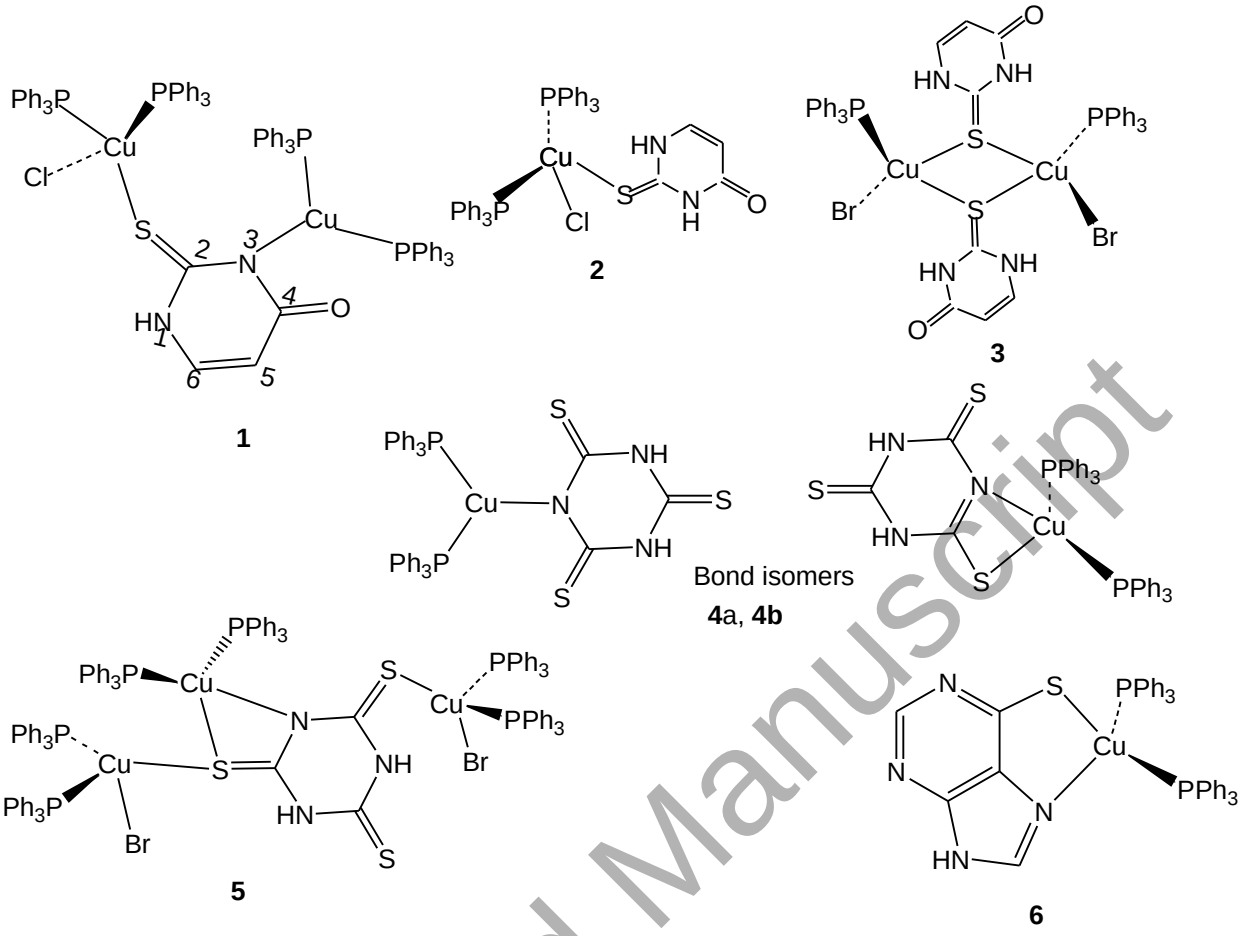


Chart 2. The structures **1, 2-6**[29,30]

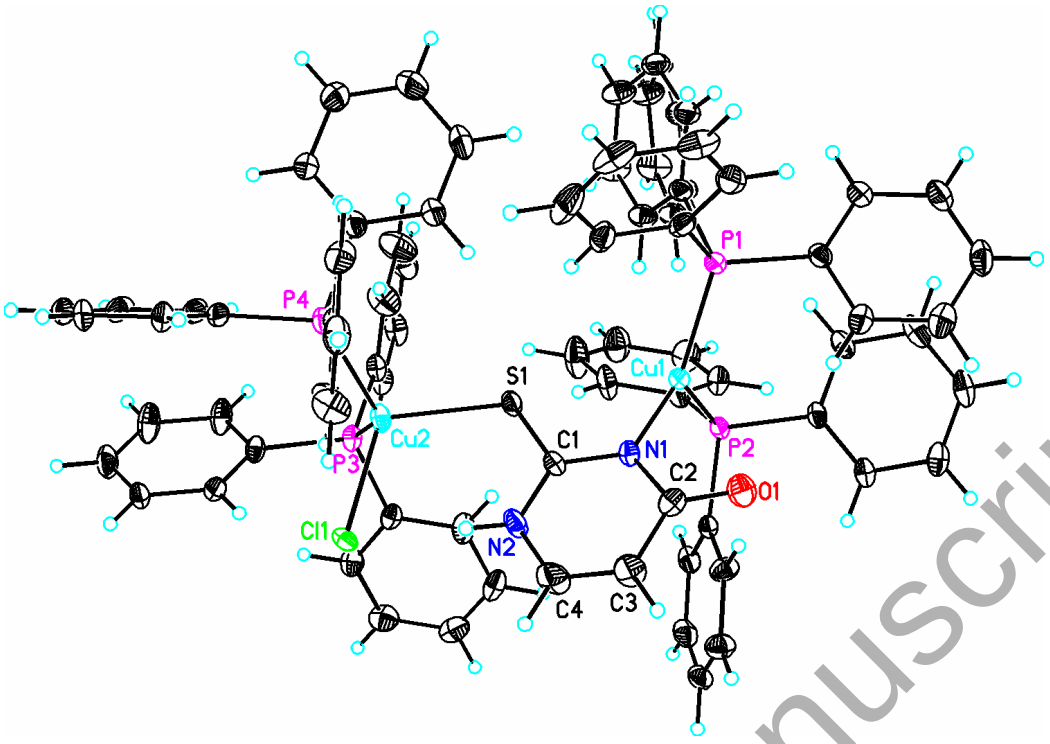


Figure 1. Molecular structure of $[Cu(PPh_3)_2(\mu\text{-}N,S\text{-}tucH)Cu(PPh_3)_2Cl]$ **1a**, the major component.

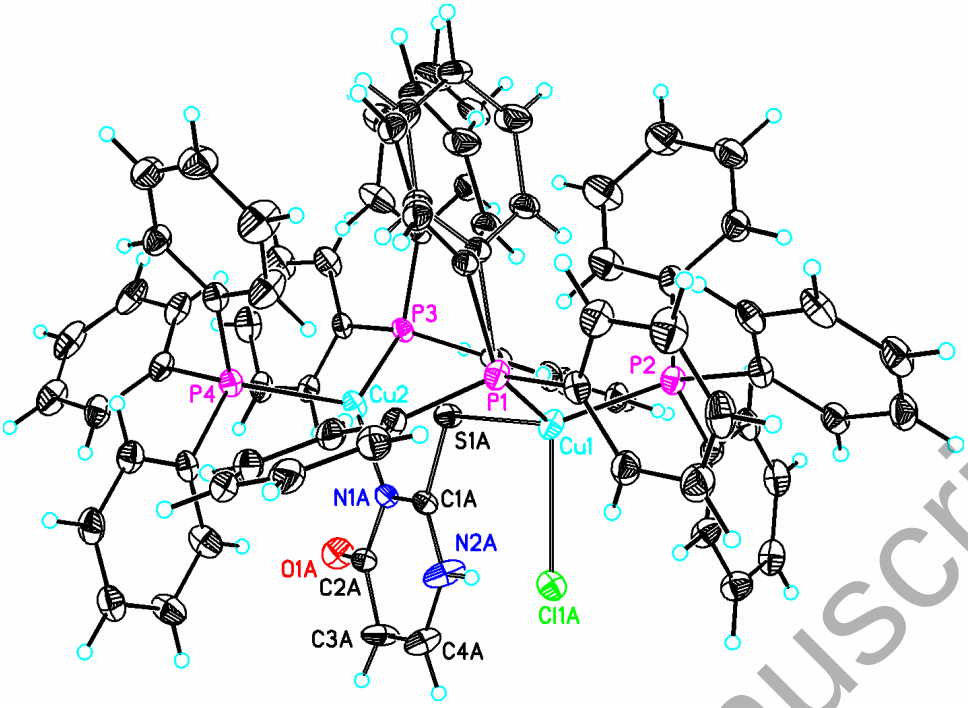


Figure 2. Molecular structure of $[Cu(PPh_3)_2(\mu\text{-}N,S\text{-}tucH)Cu(PPh_3)_2Cl]$ **1b**, the minor component.

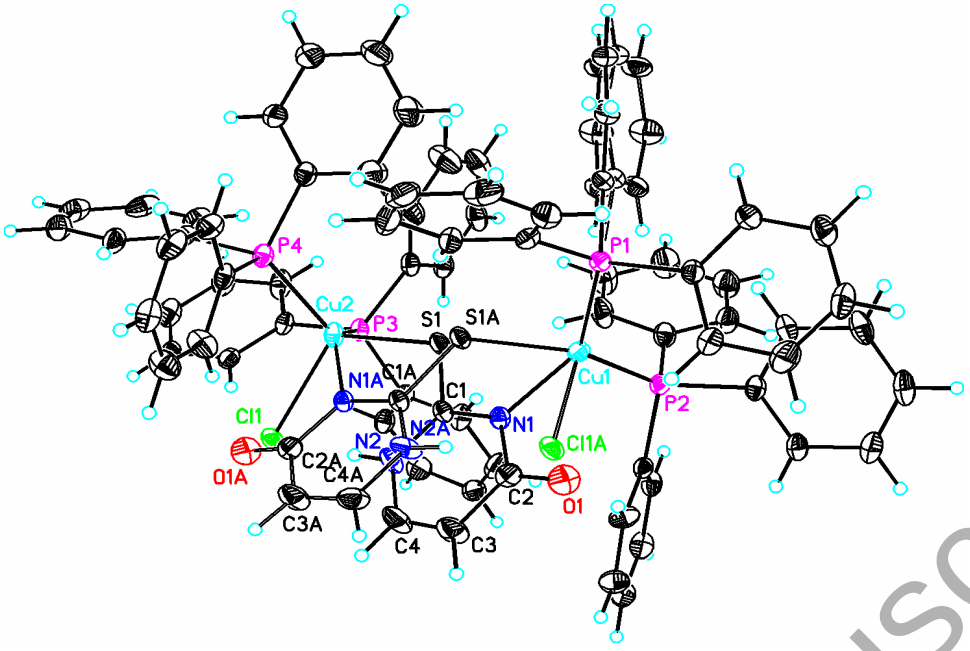


Figure 3. The overlapping major- minor components of [Cu(PPh_3)$_2$(μ-N,S- tucH)Cu(PPh_3)$_2$Cl] **1**.

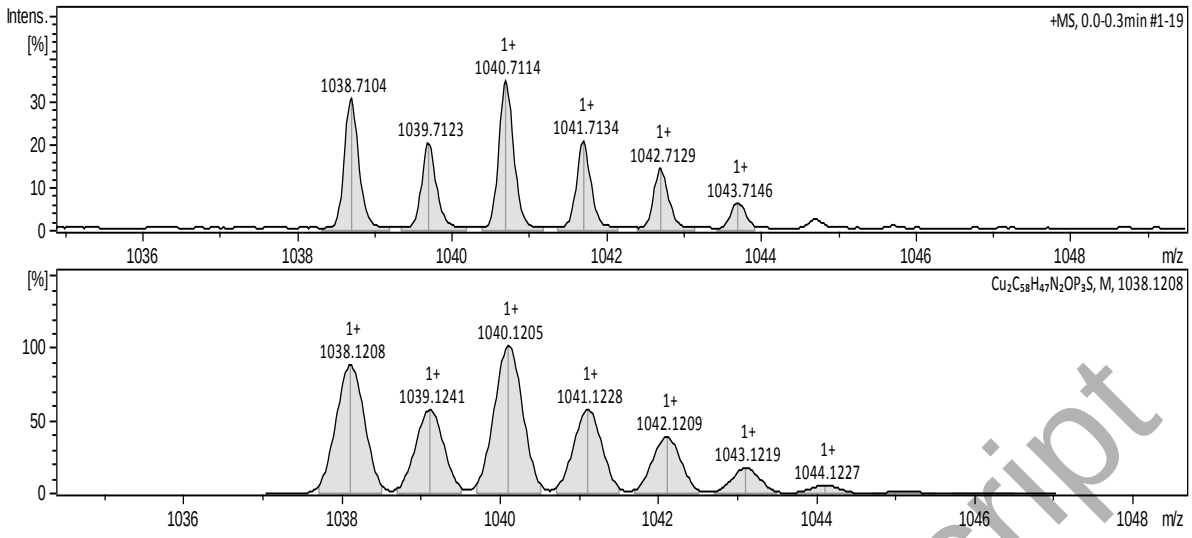


Figure 4. ESI mass spectrum of $[Cu_2(PPh_3)_3(\mu\text{-N,S-tuc})]^+$ species (A) ($Cu_2C_{58}H_{47}N_2OP_3S$; m/z = calc. 1038.12, obsd 1038.71) with isotopic pattern (**1**).

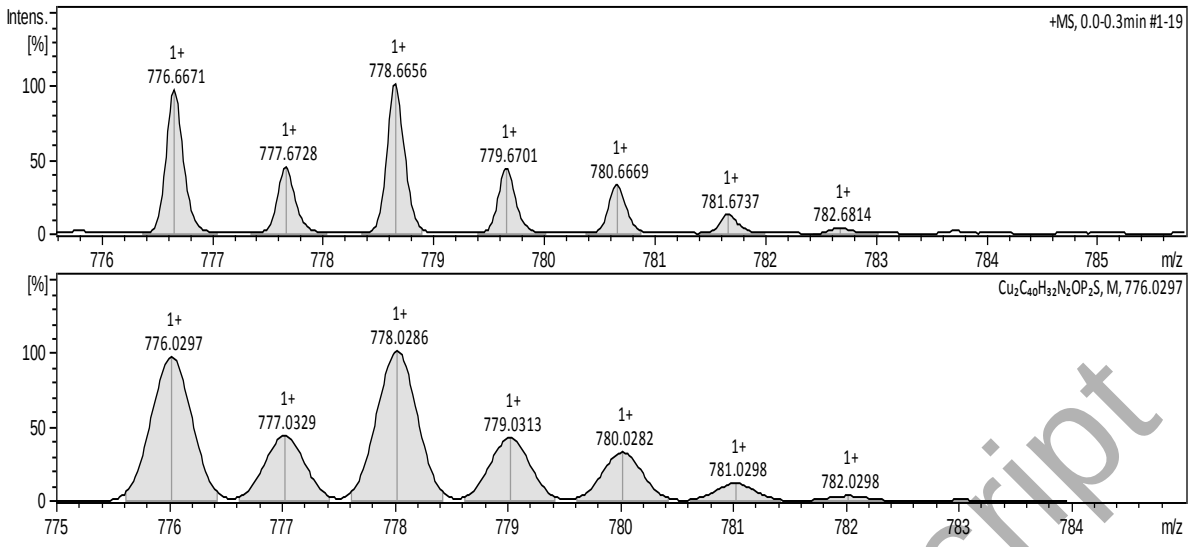


Figure 5. ESI mass spectrum of $[Cu_2(PPh_3)_2(\mu\text{-}N,S\text{-}tuc)]^+$ species (B) ($Cu_2C_{40}H_{32}N_2OP_2S$; m/z = calc, 776.02, obsd. 776.66) with isotopic pattern (**1**)

Table 1. Crystal data for 1.

T(K)	**1** 173(2) K		
Empirical formula	$C_{76}H_{63}ClCu_2N_2OP_4S$	V(Å^3)	6622.2(5)
M	1338.75	Z	4
λ(Å)	0.71073	D_{calcd}(g cm^{-3})	1.343
Crystal system	Monoclinic	μ(mm^{-1})	0.857
Space group	Ia	F(000)	2768
Unit cell Dimensions		Reflns collected	22103
a(Å)	22.2638(9)	Unique reflns	15703 (R_{int}= 0.0302)
b(Å)	11.8050(4)	Data / restraints / parameters	15703 / 110 / 688
c(Å)	27.4455(10)	Reflns.with [I>2σ(I)]	12328
α(°)	90	R Indices, R_1	0.0480
		wR_2	0.1042
β(°)	113.358(5)	R indices (all data)	
		R_1	0.0699
		wR_2	0.1192
γ(°)	90	Largest diff. peak and hole	0.567, -0.598 e.Å^{-3}

.

Table 2. Important bond lengths (Å) and angles (°) of 1

Major component (77%)			
Cu1 – N1	1.928(5)	Cu2 – P3	2.2658(13)
Cu1 – P1	2.2335(14)	Cu2 – P4	2.2912(15)
Cu1 – P2	2.2666(14)	Cu2 – S1	2.368(2)
O1 – C2	1.202(7)	Cu2 –Cl1	2.4471(18)
		S1 – C1	1.708(5)
P1–Cu1–P2	122.91(5)	N1–Cu1–P1	122.78(16)
N1–Cu1–P2	112.81(16)		
P3–Cu2–Cl1	102.03(6)	P3–Cu2–P4	120.16(5)
P3–Cu2–S1	111.08(6)	P4–Cu2–Cl1	108.28(6)
P4–Cu2–S1	107.29(6)	S1–Cu2–Cl1	107.26(6)
Minor component (23%)			
Cu1–S1A	2.431(8)	Cu2 – P3	2.2658(13)
Cu1 – P1	2.2335(14)	Cu2 – P4	2.2912(15)
Cu1 – P2	2.2666(14)	Cu2– N1A	1.768(14) .
Cu1–Cl1A	2.597(7)	O1A–C2A	1.203(17)
S1A–C1A	1.697(14)		
P2–Cu1–Cl1A	112.98(15)	P1–Cu1–P2	122.91(5)
P1–Cu1–S1A	109.35(18)	P1–Cu1–Cl1A	97.86(14)

P2–Cu1–S1A	109.17(19)	S1A–Cu1–Cl1A	102.3(2)
P3–Cu2–P4	120.15(5)	N1A–Cu2–P3	129.9(6)
N1A–Cu2–P4	108.7(6)		

Table 3. Antimicrobial activity[(a-c)] of 1-6.

Ligand/complex	*S. aureus* (MTCC 740)	*S. flexneri* (MTCC 1457)	*E. faecalis* (MTCC 439)	*E. coli* (MTCC 119)
$[Cu_2(PPh_3)_4(\mu\text{-}N,S\text{-}tucH)Cl]$**1** [This work]	15	21	16	NA[e]
$[CuCl(k^1S\text{-}tucH_2)(PPh_3)_2]$ **2** [29]	12	17	NA[e]	NA[e]
$[Cu_2Br_2(\mu\text{-}S\text{-}tucH_2)_2(PPh_3)_2]\cdot 2CHCl_3$ **3** [30]	14	16	NA[e]	NA[e]
$[Cu(tmtH_2)(PPh_3)_2]$ **4** [29]	15	ND	NA[e]	ND
$[Cu_3Br_2(k^1\text{-}N,k^1\text{-}S,\mu\text{-}S\text{-}tmtH_2)(PPh_3)_6]$ **5** [29]	18	ND	NA[e]	ND
$[Cu(k^1\text{-}N,k^1\text{-}S\text{-}purSH)(PPh_3)_2]$ **6** [29]	15	ND	NA[e]	ND
2-Thiouracil	NA[e]	15	14	16
H_3tmt	14	ND	NA[e]	ND
$purSH_2$	NA[e]	ND	NA[e]	ND
Gentamicin[d]	26	34.5	27	30.5

[a] All measurements are in mm diameter of the inhibition zone. [b] The standard deviation varied in the range 0-1 based on three readings. [c] Studies were made in dmso. [d] Commercially available antimicrobial agent and it acts as positive control against bacteria . [e]NA- not active; ND-not determined.