



SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL STUDY OF COPPER (II) COMPLEXES OF THIOSEMICARBAZONES

(Sintesis, Pencirian dan Kajian Antibakteria Kompleks Kuprum (II) Tiosemikarbazon)

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Abstract

Reaction of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ with 6-(3-thienyl) pyridine-2-carboxaldehyde-⁴N-ethyl thiosemicarbazone, (HL^1) and 6-(3-thienyl) pyridine-2-carboxaldehyde-⁴N-phenylthiosemicarbazone, (HL^2) in ethanol produced the compounds $[\text{Cu}(\text{L}1)\text{Cl}]_2 \cdot 2\text{H}_2\text{O}$ (1) and $[\text{Cu}(\text{HL})\text{Cl}_2]$ (2) respectively. The ligands and complexes were characterized by elemental analysis (C, H, N, and S), FT-IR, UV-visible, magnetic susceptibility and molar conductance. The thiosemicarbazone is a tridentate ligand coordinated via the pyridine nitrogen atom, the azomethine nitrogen atom and the sulfur atom. The thiosemicarbazones may coordinate as neutral or anionic ligands. The physicochemical and spectral data indicate a square pyramidal geometry for copper (II) atoms. The thiosemicarbazone ligands show moderate activity against bacteria MRSA and *B.subtilis*, while the copper(II) complexes were very active against MRSA, *B.subtilis* and *B.thuringiensis* and show moderate activity against *S.aureus* and *E.coli*.

Keywords: thiosemicarbazone, Cu (II) complexes, antibacterial study

Abstrak

Tindak balas garam $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ dengan 6- (3-thienyl) piridina-2-karboksilaldehid-⁴N-etil tiosemikarbazon, (HL^1) dan 6- (3-thienyl) piridina-2- karboksilaldehid-⁴N-feniltiosemikarbazon, (HL^2) dalam etanol menghasilkan masing-masing $[\text{Cu} (\text{L}1) \text{Cl}]_2 \cdot 2\text{H}_2\text{O}$ (1) dan $[\text{Cu} (\text{HL})\text{Cl}_2]$ (2). Ligan dan kompleks telah dicirikan dengan analisis unsur (C, H, N, dan S), FT-IR, UV-nampak, kerentanan magnet dan kekonduksian molar. Tiosemikarbazon adalah ligan tridentat berkoordinat melalui atom nitrogen piridina, atom nitrogen azometina dan ligan atom sulfur. Tiosemikarbazon boleh berkoordinat sebagai ligan neutral atau anionik. Data fizikokimia dan spektrum menunjukkan geometri piramid segiempat sama untuk atom kuprum(II). Ligan tiosemikarbazon menunjukkan aktiviti sederhana terhadap bakteria MRSA dan *B.subtilis*, manakala kompleks kuprum(II) sangat aktif terhadap MRSA, *B.subtilis* dan *B.thuringiensis* dan menunjukkan aktiviti sederhana terhadap *S.aureus* dan *E.coli*.

Kata kunci: tiosemikarbazon, Cu (II) kompleks, kajian anti-bakteria

Introduction

Thiosemicarbazones, with the general formula $\text{R}_1\text{R}_2\text{C}=\text{N}-\text{NH}-\text{C}=\text{S}-\text{NR}_3\text{R}_4$ are one of the most important nitrogen sulfur donor ligands. Owing to the presence of the $-\text{NH}-\text{C}=\text{S}$ functional group, thiosemicarbazones exhibit thione-thiol tautomerism and can bind to the metal ion either in the anionic thiolate form or in the neutral thione form.

Generally thiosemicarbazones coordinate as bidentate ligand via azomethine nitrogen and thione/thiolate sulfur but when additional coordination functionality is present in the proximity of donating centers, the ligands will coordinate in a tridentate manner. Due to their wide range of biological application such as antibacterial, antifungal, antioxidants and anticancer agents [1-6], heterocyclic thiosemicarbazones and their metal complexes still have considerable attention. The biological activities of thiosemicarbazones are based on the parent aldehyde or ketone. In some cases the biological activities increased with metal complexation rather than the parent ligand. In this paper, we describe the synthesis and spectroscopic characterization of novel thiosemicarbazone ligands and their copper (II) complexes as part of our ongoing studies on the synthesis and properties of thiosemicarbazones derivatives [7-9].

Materials and Methods

General Procedure

All chemicals are reagent grade and used as commercially purchased without further purification. The elemental analysis was carried out on a Flash EA 1112 CHNS-O Analyzer. Melting points were determined in an open capillary tube using an Electrothermal 9100 Digital Melting Point apparatus. Infrared spectra were recorded as KBr discs, using a Perkin –Elmer FT-IR model GX Infrared Spectrophotometer, UV spectra were obtained as DMSO solutions with UV-2450 version UV-VIS spectrophotometer. Magnetic moment of the complexes at 300 K was measured on a Sherwood Scientific Magnetic Susceptibility Balance. Conductance values were obtained with a EUTECH COND 610 Conductometer at 298 K from 10^{-3} mol L⁻¹ solutions of complexes in DMSO. The ¹H and ¹³C nuclear magnetic resonance spectra were recorded using the BRUKER FT-NMR 600 MHz Cryo-Prob spectrometer, using d6-DMSO as a solvent and tetramethylsilane as an internal standard.

Synthesis of the ligands

The ligands were synthesized by the condensation of 6-(3-thienyl) pyridine-2-carboxaldehyde with 4- ethyl-3-thiosemicarbazide or 4-phenylthiosemicarbazide in 1:1 molar ratio using absolute ethanol as the reaction medium. The resulting precipitate was filtered, washed with ethanol and dried over silica gel.

6-(3-thienyl) pyridine-2-carboxaldehyde-⁴N-ethyl thiosemicarbazone, (HL¹)

Colour: yellow. Yield: 98%. Melting point: 223-225 °C. Elemental Anal. Calc. for C₁₃H₁₄N₄S₂: C, 53.77; H, 4.86; N, 19.29; S, 22.08. Found: C, 54.02; H, 4.17; N, 19.22; S, 21.92%. Molecular weight: 290.41. Main IR peaks (KBr, cm⁻¹): ν(N-H) 3392w, 3271s; ν(C=N) 1607s; ν(C=S) 884m; py(ip) 610m. UV-VIS (nm): 276, 335. ¹H NMR (DMSO-d₆, ppm): 8.80 (t, 1H, N(4)H); 11.78 (s, 1H, N(3)H); 8.14 (s, 1H, C(10)H); 7.68-8.25 (m, 6H, C-H aromatic); 3.61 (q, 2H, C-H aliphatic); 1.16 (t, 3H, C-H aliphatic); ¹³C NMR (DMSO-d₆, ppm): 177.33 (C=S); 141.53 (C=N); 119.20-153.19 (aromatic); 14.98, 38.87 (aliphatic).

6-(3-thienyl) pyridine-2-carboxaldehyde-⁴N-phenyl thiosemicarbazone, (HL²)

Colour: yellow. Yield: 92%. Melting point: 141-143 °C. Elemental Anal. Calc. for C₁₇H₁₄N₄S₂: C, 60.33; H, 4.17; N, 16.55; S, 18.95. Found: C, 59.76; H, 3.47; N, 15.90; S, 19.63%. Molecular weight: 338.45. Main IR peaks (KBr, cm⁻¹): ν(N-H) 3331w, 3230m; ν(C=N) 1611m; ν(C=S) 892m; py(ip) 613m. UV-VIS (nm): 268, 349. ¹H NMR (DMSO-d₆, ppm): 10.33 (s, 1H, N(4)H); 12.16 (s, 1H, N(3)H); 8.28 (s, 1H, C(10)H); 7.23-8.39 (m, 11H, C-H aromatic). ¹³C NMR (DMSO-d₆, ppm): 176.97 (C=S); 142.99 (C=N); 119.64-153.17 (aromatic).

Synthesis of complexes: Synthesis of [Cu(L¹)Cl]₂.2H₂O(1)

CuCl₂.2H₂O (0.17 g, 1 mmol) solid was added to a solution of ethanol (60 ml) of HL¹ (0.29 g, 1 mmol). The mixture was heated under reflux for 1 hour. Reddish brown precipitate formed was filtered, washed with ethanol, ether and dried in vacuo over silica gel. Yield: 37%. Melting point: 217-218. Elemental Anal. Calc. for C₂₆H₃₀N₈S₄O₂Cl₂Cu₂: C, 38.42; H, 3.72; N, 13.79; S, 15.78. Found: C, 38.32; H, 3.27; N, 13.30; S, 15.81%. Molecular weight: 812.83. Main IR peaks (KBr, cm⁻¹): ν(N-H) 3330s; ν(C=N) 1566m; ν(C=S) 889w; py(ip) 629m. UV-VIS (nm): 270, 331, 419, 620. ¹H NMR (DMSO-d₆, ppm): 8.75 (t, 1H, N(4)H); 8.14 (s, 1H, C(10)H); 7.58-8.21 (m, 6H, C-H aromatic); 3.61 (q, 2H, C-H aliphatic); 1.18 (t, 3H, C-H aliphatic); ¹³C NMR (DMSO-d₆, ppm): 177.42 (C=S); 140.84 (C=N); 119.40-152.85 (aromatic); 14.85, 38.91 (aliphatic).

Synthesis of [Cu(HL²)Cl₂] (2)

CuCl₂·2H₂O (0.17 g, 1mmol) solid was added to a solution of an ethanol (70 ml) of HL² (0.34 g, 1mmol). The mixture was heated under reflux for 1 hour. Dark green precipitate formed was filtered, washed with ethanol, ether and dried in vacuo over silica gel. Yield: 62%. Melting point: 186-187. Elemental Anal. Calc. for C₁₇H₁₄N₄S₂Cl₂Cu : C, 43.18; H, 2.98; N, 11.85; S, 13.56. Found: C, 43.38; H, 2.47; N, 11.61; S, 14.42%. Molecular weight: 472.90. Main IR peaks (KBr, cm⁻¹): ν(N-H) 3269vs, 3187w; ν(C=N) 1598s; ν(C=S) 889m; py(ip) 631m. UV-VIS (nm): 261, 328, 415, 620.

Antibacterial Activity

The antibacterial activity of the ligands and copper (II) complexes was evaluated by measuring the diameters of the inhibition zone exhibited by the tested compounds (at concentration 20mg/ml in DMSO) using disc diffusion method. The test organisms chosen for antibacterial activity were five Gram-positive *MRSA*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus thuringiensis*, and five Gram-negative *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Enterobacter aerogenes*. Vancomycin (30µg/disc), Gentamicin (10µg/disc) and Chloramphenicol (10µg/disc) were used as standard drugs.

Results and Discussion

The isolated solid complexes are stable in air and their molar conductivities are in the range that indicates a non-electrolytic nature. The highest molar conductivity in complex **1** is probably due to partial substitution by the solvent DMSO for the chloro ligand [10]. The magnetic susceptibilities at room temperature showed that complex **1** is diamagnetic which indicated that the single electron in each copper (II) ion has been spin-paired due to the strong Cu (II) - Cu (II) interaction, whereas complex **2** is paramagnetic and has a magnetic moment value 1.7 B.M which points to one unpaired electron for copper (II) complex[11,12].

Infrared spectra

The IR spectral data of the ligands and copper (II) complexes are listed in Table 1 and 2 respectively. The ν(-NH₂) and ν(-NH-) were observed at the range of 3331-3392 and 3230-3271 cm⁻¹ respectively, which shifted to lower wavenumbers for the copper (II) complexes. The band at 3172 cm⁻¹ in the spectrum of HL¹ due to ν(-NH-) is absent in the spectrum of complex **1** providing strong evidence for deprotonation of the thiosemicarbazone and chelation in the thiolate form [13]. The ν(C=N) observed in the range 1607- 1611 cm⁻¹ in the spectrum of the free bases shifts to 1566-1598 cm⁻¹ in the spectra of the copper(II) complexes, indicating azomethine nitrogen coordination [11, 14]. The ν(C=S) absorption at 884-892 cm⁻¹ in the spectrum of the uncomplexed thiosemicarbazone is observed at 889 cm⁻¹ in the complexes indicating coordination of thiolate/thione sulfur [15]. The pyridine in-plane deformation mode at 610-613 cm⁻¹ in the spectra of free thiosemicarbazones ligand shifts to 629-631 cm⁻¹ in those of the complexes, suggesting coordination of the heteroaromatic nitrogen [16]. In addition, new absorption at 460-477 cm⁻¹ and 228 - 242cm⁻¹ have been assigned to ν(Cu-N) and ν(Cu-N_{py}) respectively, and bands in the 348-364 cm⁻¹ range have been attributed to ν (Cu-S) [11, 17]. The band at 316-324 cm⁻¹ has been assigned to ν(Cu-Cl) [18].

Table 1. IR spectral data of the ligands and copper (II) complexes (cm⁻¹)

Compound	ν(-NH ₂)	ν(-NH-)	ν(C=N)	ν(C=S)	Py (ip)
HL ¹	3392	3271	1607	884	610
HL ²	3331	3230	1611	892	613
[Cu(L ¹)Cl] ₂ ·2H ₂ O (1)	3330	-	1566	889	629
[Cu(HL ²)Cl ₂] (2)	3269	3187	1598	889	631

Table 2. Far-IR spectral data of the ligands and copper (II) complexes (cm⁻¹)

Compound	$\nu(\text{Cu-N})$	$\nu(\text{Cu-S})$	$\nu(\text{Cu-N}_{\text{py}})$	$\nu(\text{Cu-Cl})$
[Cu(L ¹)Cl] ₂ .2H ₂ O (1)	460	348	242	316
[Cu(HL ²)Cl ₂] (2)	477	364	228	324

Electronic spectra

The significant electronic spectral bands of the ligands and copper (II) complexes are presented in Table 3. In the electronic spectra of the free thiosemicarbazone ligands (DMSO) the ring $\pi - \pi^*$ transition at 36232-37313 cm⁻¹ are not significantly changed upon complexation. The absorption at 28653-29851cm⁻¹ is assigned to n- π^* transition within the thiosemicarbazone moiety involving mainly C=N and C=S is shifted to higher energies in the spectra of the complexes [19]. The band appeared at 23866 cm⁻¹ and 24096 cm⁻¹ for complex **1** and **2**, respectively is assigned to a charge transfer transition [20]. The $d-d$ transition was found at 16129 cm⁻¹ suggestive of a square-pyramidal geometry for the present metal complexes similar to the one observed for many analogous of thiosemicarbazones compounds [21].

Table 3. Electronic spectral data (cm⁻¹) of thiosemicarbazone ligands and copper (II) complexes

Compound	$\pi - \pi^*$	n - π^*	CT	$d-d$
HL ¹	36232	29851	-	-
HL ²	37313	28653	-	-
[Cu(L ¹)Cl] ₂ .2H ₂ O (1)	37037	30211	23866	16129
[Cu(HL ²)Cl ₂] (2)	38314	30488	24096	1629

¹H and ¹³C NMR spectra

The ¹H and ¹³C NMR spectral data of the ligands and copper (II) complexes are listed in Table 4 and 5 respectively. The N(4)-H signal appears at 8.80 and 10.33 ppm for ligands HL¹ and HL², respectively. In the spectra of complex **1**, this signal is shifted upfield [22]. The N(3)-H proton signal was observed at 11.78 ppm for ligand HL¹disappeared in the ¹H-NMR spectra of the complex **1**. This is the evidence of ligand deprotonation during metal chelating [23],the aromatic ring protons which were observed as multiplets between 7.68-8.25 ppm exhibit small changes upon complexation [24]. A multiplet that found at 3.61 ppm together with a triplet at 1.16 ppm which represents protons of the ethyl substituent on N(4) in complex **1** is appeared nearly as the same region as of its corresponding free ligand [25].

Table 4. ¹H NMR spectral data (ppm) of the ligands and copper (II) complexes

Compound	N(4)H	N(3)H	C(10)H	C-H Aromatic	C-H Aliphatic
HL ¹	8.80	11.78	8.14	7.68-8.25	3.61,1.16
HL ²	10.33	12.16	8.28	7.23-8.39	-
[Cu(L ¹)Cl] ₂ .2H ₂ O (1)	8.75	-	8.14	7.58-8.21	3.61,1.18

Table 5. ^{13}C NMR spectral data (ppm) of the ligands and copper (II) complexes

Compound	C=S	C=N	Aromatic	Aliphatic
HL ¹	177.33	141.53	119.20-153.19	14.98,38.87
HL ²	176.97	142.99	119.64-153.17	-
[Cu(L ¹)Cl] ₂ .2H ₂ O (1)	177.42	140.84	119.40-152.85	14.85,38.91

Antibacterial study

The results of antibacterial study were given in Table 6 and 7. Based on the observed inhibition zones, it was found that the ligands and the complexes are inactive against some of the tested bacteria. Furthermore, the ligands showed a weak activity against G (+) *Bacillus thuringiensis* and *Staphylococcus aureus*. Also the ligands exhibited moderate activity against G (+) *MRSA* and *Bacillus subtilis*. Both of the complexes showed moderate activity against G (+) *Staphylococcus aureus*. Furthermore, moderate activity was shown by the complex 1 against G (-) *Escherichia coli*. In the case of G (+) *MRSA*, *Bacillus subtilis* and *Bacillus thuringiensis* the complexes were found to be very active, where as some activity was displayed by complex 1 against G(-) *Proteus vulgais* and *Salmonella typhimurium*. On the other hand, a weak activity was recorded for complex 2 against G (-) *Salmonella typhimurium* and G (+) *Bacillus thuringiensis*.

Table 6. Antibacterial activity data of ligands and copper (II) complexes, G (+)

Compound	Conc. mg/ml	Zone of inhibition diameter (mm)				
		MRSA	S.epid	S. aur	B. subt	B.thur
HL ¹	20	8	6	10	9	6
HL ²	20	8	6	8	8	8
[Cu(L ¹)Cl] ₂ .2H ₂ O (1)	20	11	8	11	13	15
[Cu(HL ²)Cl ₂] (2)	20	10	8	10	13	9
Vancomycin		18	-	-	-	-
Gentamicin		-	20	25	-	-
Chloramphenicol		-	-	-	20	25

Table 7. Antibacterial activity data of ligands and copper (II) complexes, G (-)

Compound	Conc. mg/ml	Zone of inhibition diameter (mm)				
		P. aerug	E.coli	P. vulg	S. typh	E. aerog
HL ¹	20	6	8	6	6	7
HL ²	20	6	6	6	6	6
[Cu(L ¹)Cl] ₂ .2H ₂ O (1)	20	6	11	11	10	7
[Cu(HL ²)Cl ₂] (2)	20	6	8	6	9	7
Vancomycin		-	-	-	-	-
Gentamicin		20	-	-	-	-
Chloramphenicol		-	24	30	30	26

The structure of ligands used in this study were illustrated in Figure 1 and 2 respectively. Meanwhile proposed structure of complex as showed in Figure 3 and 4 respectively.

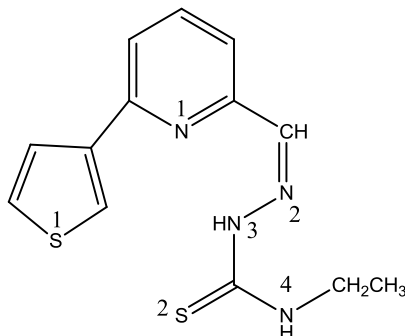


Figure 1. 6-(3-thienyl) pyridine-2-carboxaldehyde-⁴N-ethyl thiosemicarbazone, (HL¹)

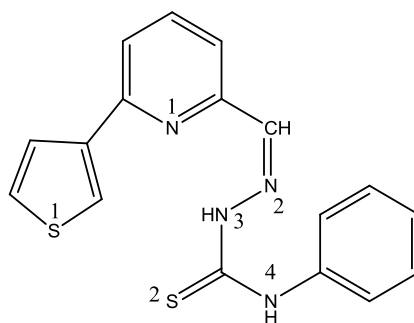


Figure 2. 6-(3-thienyl) pyridine-2-carboxaldehyde-⁴N-phenyl thiosemicarbazone, (HL²)

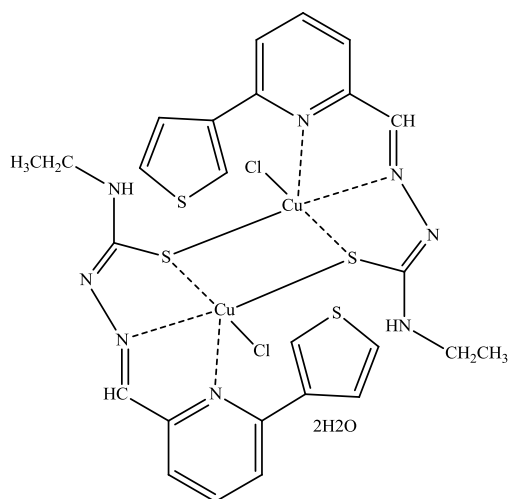


Figure 3. Proposed structure of complex 1

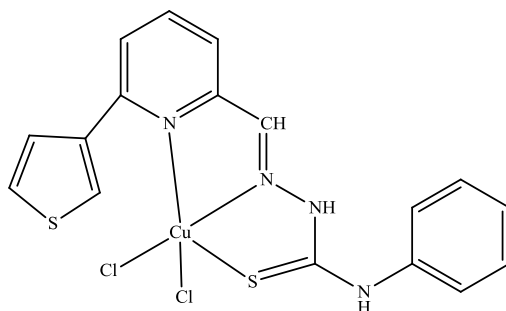


Figure 4. Proposed structure of complex 2

Conclusion

It may be concluded that the ligands behave as tridentate chelating agent coordinating through azomethane nitrogen, thiolate/thion sulfur and pyridine nitrogen. The magnetic and electronic spectra studies suggest a square-pyramidal geometry for the present metal complexes. Copper (II) complexes showed moderate activity against G (+) *Staphylococcus aureus*. Furthermore, a moderate activity was shown by the complex **1** against G (-) *Escherichia coli*. The complexes were found to be very active against G (+) *MRSA*, *Bacillus subtilis* and *Bacillus thuringiensis*.

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References

1. Aly, M. M., Mohamed, Y. A., El-Bayouki, K. A., Basyouni, W. M. and Abbas, S. Y. (2010). Synthesis of Some New 4(3h)-Quinazolinone-2-Carboxaldehyde Thiosemicarbazones and Their Metal Complexes and a Study on Their Anticonvulsant, Analgesic, Cytotoxic and Antimicrobial Activities - Part-1. *European Journal of Medicinal Chemistry*, 45(8): 3365-3373
2. Vinuelas-Zahinos, E., Luna-Giles, F., Torres-Garcia, P. and Fernandez-Calderon, M. C. (2011). Co(III), Ni(II), Zn(II) and Cd(II) Complexes with 2-Acetyl-2-Thiazoline Thiosemicarbazone: Synthesis, Characterization, X-Ray Structures and Antibacterial Activity. *European Journal of Medicinal Chemistry*, 46(1): 150-159.
3. Halder, S., Paul, P., Peng, S.-M., Lee, G.-H., Mukherjee, A., Dutta, S., Sanyal, U. and Bhattacharya, S. (2012). Benzaldehyde Thiosemicarbazone Complexes of Platinum: Syntheses, Structures and Cytotoxic Properties. *Polyhedron*, 45(1): 177-184.
4. Liu, Z. C., Wang, B. D., Yang, Z. Y., Li, Y., Qin, D. D. and Li, T. R. (2009). Synthesis, Crystal Structure, DNA Interaction and Antioxidant Activities of Two Novel Water-Soluble Cu²⁺ Complexes Derived from 2-Oxo-Quinoline-3-Carbaldehyde Schiff-Bases. *European Journal of Medicinal Chemistry*, 44(11): 4477-4484.
5. Sampath, K., Sathiyaraj, S., Raja, G. and Jayabalakrishnan, C. (2013). Mixed Ligand Ruthenium (III) Complexes of Benzaldehyde 4-Methyl-3-Thiosemicarbazones with Triphenylphosphine/Triphenylarsine Co-Ligands: Synthesis, DNA Binding, DNA Cleavage, Antioxidative and Cytotoxic Activity. *Journal of Molecular Structure*, 1046(0): 82-91.
6. Chandra, S., Bargujar, S., Nirwal, R. and Yadav, N. (2013). Synthesis, Spectral Characterization and Biological Evaluation of Copper (II) and Nickel (II) Complexes with Thiosemicarbazones Derived from a Bidentate Schiff Base. *Spectrochimica Acta A: Molecular Biomolecular Spectroscopy*, 10691-10698.
7. Dzulkifli, N. N., Farina, Y., Yamin, B. M., Babaa, I. and Tiekink, E. R. T. (2011). 3-[(E)-(4-Chloro-Benzyl-Idene)Amino]-1-Phenyl-Thio-Urea. *Acta Crystallographica Section E Structure Report Online*, 67(Pt 4): o872.
8. Normaya, E., Farina, Y., Halim, S. N. A. and Tiekink, E. R. T. (2011). 3-(2-Hydroxy-Phen-Yl)-1-[(E)-[1-(Pyrazin-2-Yl)Ethyl-Idene]Amino]-Thio-Urea Monohydrate. *Acta Crystallographica Section E Structure Report Online*, 67(Pt 4): o943-944.

9. Kayed, S. A. F., Farina, Y., Kassim, M. and Simpson, J. (2008). (E)-2-[1-(1-Benzothio-Phen-3-Yl)Ethyl-Idene]Hydrazinecarbothio-Amide Methanol Hemisolvate. *Acta Crystallographica Section E Structure Report Online*, 64(Pt 6): o1022-1023.
10. Min Wang, L.-F. W., Yi-Zhi Li and Qin-Xi Li. (2001). Antitumour Activity of Transition Metal Complexes with the Thiosemicarbazone Derived from 3-Acetylbulliferone. *Transition Metal Chemistry*, 26 (307-310).
11. Labisbal, E., Haslow, K. D., Sousa-Pedraes, A., Valdés-Martínez, J., Hernández-Ortega, S. and West, D. X. (2003). Copper (II) and Nickel (II) Complexes of 5-Methyl-2-Hydroxyacetophenone N(4)-Substituted Thiosemicarbazones. *Polyhedron*, 22(20): 2831-2837.
12. Sen, S., Shit, S., Mitra, S. and Batten, S. R. (2007). Structural and Spectral Studies of a New Copper (II) Complex with a Tridentate Thiosemicarbazone Ligand. *Structural Chemistry*, 19(1): 137-142.
13. Ilies, D.-C., Pahontu, E., Shova, S., Gulea, A. and Rosu, T. (2013). Synthesis, Characterization and Crystal Structures of Nickel(II), Palladium(II) and Copper(II) Complexes with 2-Furaldehyde-4-Phenylthiosemicarbazone. *Polyhedron*, 51(0): 307-315.
14. John, R. P., Sreekanth, A., Kurup, M. R. P. and Fun, H.-K. (2005). Chelating Behavior of 2-Hydroxyacetophenone N(4)-Disubstituted Thiosemicarbazones: Facile Formation of Mn(IV) Complexes – X-Ray Structure, Epr and Cyclic Voltammetric Studies. *Polyhedron*, 24(5): 601-610.
15. Mari'a C. Rodri'Guez-Argu'Elles, E. C. L. P.-S., Jesu' S Sanmarti'N, and P. Pelagatti, F. Z. D. (2005) Copper Complexes of Imidazole-2-, Pyrrole-2- and Indol-3-Carbaldehyde Thiosemicarbazones: Inhibitory Activity against Fungi and Bacteria. *Journal of Inorganic Biochemistry*, 99:2231–2239.
16. El-Ayaan, U., Youssef, M. M. and Al-Shihry, S. (2009). Mn(II), Co(II), Zn(II), Fe(III) and U (VI) Complexes of 2-Acetylpyridine 4n-(2-Pyridyl) Thiosemicarbazone (Hapt); Structural, Spectroscopic and Biological Studies. *Journal of Molecular Structure*, 936(1-3): 213-219.
17. Joseph, M., Kuriakose, M., Kurup, M. R. P., Suresh, E., Kishore, A. and Bhat, S. G. (2006). Structural, Antimicrobial and Spectral Studies of Copper (II) Complexes of 2-Benzoylpyridine N(4)-Phenyl Thiosemicarbazone. *Polyhedron*, 25(1): 61-70.
18. Isolda C. Mendes, J. P. M., Nivaldo L. Speziali, Antonio S. Mangrich, & Beraldo, J. A. T. A. H. (2006). N(4)-Tolyl-2-Benzoylpyridine Thiosemicarbazones and Their Copper(II) Complexes with Significant Antifungal Activity. Crystal Structure of N(4)-Para-Tolyl-2-Benzoylpyridine Thiosemicarbazone. *Journal of Brazilian Chemical Society* 17(8): 1571-1577.
19. Rapheal, P. F., Manoj, E. and Prathapachandra Kurup, M. R. (2007). Copper (II) Complexes of N(4)-Substituted Thiosemicarbazones Derived from Pyridine-2-Carbaldehyde: Crystal Structure of a Binuclear Complex. *Polyhedron*, 26(4): 818-828.
20. Philip, V., Suni, V., Prathapachandra Kurup, M. R. and Nethaji, M. (2005). Novel Binuclear Copper(II) Complexes of Di-2-Pyridyl Ketone N(4)-Methyl, N(4)-Phenylthiosemicarbazone: Structural and Spectral Investigations. *Polyhedron*, 24(10): 1133-1142.
21. Afrasiabi, Z., Sinn, E., Kulkarni, P. P., Ambike, V., Padhye, S., Deobagakar, D., Heron, M., Gabbutt, C., Anson, C. E. and Powell, A. K. (2005). Synthesis and Characterization of Copper (II) Complexes of 4-Alkyl/Aryl-1,2-Naphthoquinones Thiosemicarbazones Derivatives as Potent DNA Cleaving Agents. *Inorganica Chimica Acta*, 358(6): 2023-2030.
22. Yadav, P. N., Demertzis, M. A., Kovala-Demertzi, D., Skoulika, S. and West, D. X. (2003). Palladium (II) Complexes of 4-Formylantipyrine N(3)-Substituted Thiosemicarbazones: First Example of X-Ray Crystal Structure and Description of Bonding Properties. *Inorganica Chimica Acta*, 349: 30 - 36.
23. Basu, A. and Das, G. (2011). Zn (II) and Hg (II) Complexes of Naphthalene Based Thiosemicarbazone: Structure and Spectroscopic Studies. *Inorganica Chimica Acta*, 372: 394–399.
24. Matesanz, A. I. and Souza, P. (2007). Novel cyclopalladated and coordination palladium and platinum complexes derived from α -diphenyl ethanedione bis(thiosemicarbazones): Structural studies and cytotoxic activity against human A2780 and A2780cisR carcinoma cell lines. *Journal of Inorganic Biochemistry*, 101: 1354-1361.
25. Prabhakaran, R., Sivasamy, R., Angayarkanni, J., Huang, R., Kalaivani, P., Karvembu, R., Dallemer, F. and Natarajan, K. (2011). Topoisomerase II Inhibition Activity of New Square Planar Ni (II) Complexes Containing N-Substituted Thiosemicarbazones: Synthesis, Spectroscopy, X-Ray Crystallography and Electrochemical Characterization. *Inorganica Chimica Acta*, 374: 647–653.