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Novel α -N-heterocyclic thiosemicarbazone complexes: synthesis, characterization, and antimicrobial of properties investigation†

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In this paper, eight novel α -N-heterocyclic thiosemicarbazone complexes were synthesized in search of new biologically active compounds, and characterized *via* organic elemental analysis, nuclear magnetic resonance spectroscopy, infrared spectra, thermogravimetric analysis, ultraviolet-visible spectroscopy, molar conductance and magnetic susceptibility measurements. The *in vitro* antimicrobial activity of these complexes was examined against ten disease-causing pathogens: Gram-positive bacteria (*Micrococcus luteus* ATCC9341, *Staphylococcus epidermidis* ATCC12228, *Bacillus cereus* RSKK863) and Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumonia* ATCC27853, *Enterobacter aerogenes* ATCC51342, *Salmonella typhi* H NCTC9018394, *Shigella dysenteria* NCTC2966, *Proteus vulgaris* RSKK96026) and yeast (*Candida albicans* Y-1200-NIH). The results revealed that the α -N-heterocyclic thiosemicarbazone compounds showed potent activity. It was observed that all thiosemicarbazone complexes were more susceptible to Gram-negative strains based on the presence of an electron-withdrawing substituent (-Br/-Cl/-F). It was determined that thiosemicarbazone Cu²⁺ complexes showed stronger antifungal effects.

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1 Introduction

Heterocyclic compounds are organic structures in which some carbon atoms are replaced by heteroatoms such as nitrogen, sulphur, oxygen, and phosphorus.¹ Heterocyclics have different physical and chemical properties and reactivity depending on the heteroatoms' ring size and structure.² Recently, there has been an increasing interest in heterocyclic compounds due to their various applications. The synthesis of innovative, stereo-selective, functional new heterocyclic compounds is of great interest for drug discovery and development.³ Heterocyclic compounds are used in the agrochemical industry in crop protection due to their pesticidal activities.⁴ Heterocycles are used in the pharmaceutical industry to treat some diseases (such as Alzheimer's, cancer, diabetes, circulatory diseases, and

AIDS) due to their therapeutic properties.⁵ Heterocycles bind with enzymes due to their variety of intermolecular interaction properties. These properties of heterocycles are the reasons for preference in anti-cancer drug design.⁶ Particularly, thiosemicarbazone-based heterocyclics are an important class of heterocyclic compounds because of their coordination capacity.⁷ They are important chelating ligands due to the containing potential donor atoms (deprotonated phenolic oxygen, thione/thiol sulphur, azomethine nitrogen, *etc.*).⁸ Especially, α -N-heterocyclic thiosemicarbazones, in which the thiosemicarbazone side chain is linked to an N-heterocyclic ring at the α position, are strong metal chelating agents.⁹ Heterocyclic compounds containing thiosemicarbazone have various biological, cytotoxic, and pharmacological activities. These properties of thiosemicarbazones are generally related to the presence of imine group (-N=CH-) and metal ion coordination. The coordination affects such properties as lipophilicity, drug resistance, *etc.*¹⁰⁻¹² The complexes can exhibit bioactivities not shown by the free ligands.¹³ Heterocyclic thiosemicarbazones and their metal complexes exhibit a broad range of activities such as antibacterial, antioxidant, antitumor, antimicrobial, antiviral, antifungal, antimalarial, anticonvulsant, anti-HIV, antiamebic, antiproliferative, anti-inflammatory, antidiabetic, and anticancer.¹⁴⁻¹⁸ Heterocyclic thiosemicarbazone complexes of nickel metal play an important role in the biology of microorganisms and plants, have variable binding modes, and show strong biological, antibacterial, and

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inhibitory activity.^{10,19–22} Heterocyclic thiosemicarbazone complexes of copper, which catalysed redox reactions and are essential for life and the most effective available divalent ion for binding to organic compounds, strong exhibit antibacterial, antitumor, anticancer, and antimicrobial activity.^{23–26} Thiosemicarbazone transition metal complexes have been reported as extensively effective and preferred drugs.^{27,28} The synthesis of new heterocyclic thiosemicarbazone metal complexes is important due to their biological and pharmacological properties. Therefore, novel α -N-heterocyclic thiosemicarbazone complexes were synthesized using thiosemicarbazide, carboxaldehyde, aldehyde derivatives, and metal salts.

It is predicted that the new heterocyclic thiosemicarbazone Ni²⁺ and Cu²⁺ complexes synthesized within the scope of this study will contribute to the importance of heterocyclic chemistry in the pharmaceutical industry and medicinal chemistry.

2 Experimental

2.1. Materials and measurements

All chemicals were procured from Sigma-Aldrich. Organic elemental analyses were obtained with a Thermo-Scientific Flash-2000 model elemental analyzer. IR spectra were recorded from KBr pellets using a Shimadzu IR Prestige-21 model spectrometer. ¹H-NMR spectra were measured on a Bruker Biospin brand Avance III 400 MHz model device. UV-Vis spectra were determined on a UV-1800 ENG240V, Soft model spectrophotometer. TGA analysis were carried out using a Shimadzu DTG 60H-DSC 60 model thermal analyzer. The molar conductance of complexes was measured in dimethyl sulphoxide (DMSO) at 21 °C on Conductivity 430 Lab. Magnetic measurements were obtained with a Sherwood Scientific MKI model Evans magnetic susceptibility device.

2.2. Synthesis of heterocyclic compounds containing thiosemicarbazone

All heterocyclic thiosemicarbazone Ni²⁺ and Cu²⁺ complexes were prepared with the template method (Fig. 1 and 2). Novel heterocyclic thiosemicarbazone complexes (H₁Ni, H₂Ni, H₃Ni, H₄Ni, H₁Cu, H₂Cu, H₃Cu, and H₄Cu) were synthesized by the reaction of 4-phenyl-thiosemicarbazide (4 mmol) and 2-aminothiazole-5-carboxaldehyde (4 mmol) in ethanol/DMSO mixture. The pH of the solution was adjusted to 5–5.5 with 1 mL of acetic acid. The solution was heated with stirring under reflux for 5 h at 80 °C. The ethanol solution (50 mL) of the salicylaldehyde derivatives (4 mmol) was added to the mixture and stirred for a further 5 h at 80 °C. 5-Fluoro-3-methylsalicylaldehyde, 5-bromo-salicylaldehyde, 3-chloro-5-fluorosalicylaldehyde, and 5-methylsalicylaldehyde were used as salicylaldehyde. The ethanol solution (5 mL) of the metal salts [nickel(II) chloride hexahydrate & copper(II) chloride (anhydrous)] was added dropwise to the reaction mixture and stirred by refluxing for a further 4 h at 70 °C. The mixture was evaporated at room temperature. The colored product was filtered, purified, and dried.

The structures of all heterocyclic thiosemicarbazone complexes (H₁Ni, H₂Ni, H₃Ni, H₄Ni, H₁Cu, H₂Cu, H₃Cu, and H₄Cu) were determined by spectroscopic techniques.

2.2.1 H₁Ni. Yield 0.3503 g (69%), burgundy solid, mp 192 °C. IR spectrum (KBr), ν , cm⁻¹: 3340 (OH); 829 (H₂O); 3025 (CH)_{aro.}; 1615 (CH=N); 1544 (CH=N)_{lyzi.}; 1495 (C=C); 1210, 819 (C=S); 750 (C–S–C); 1003 (N–N); 3243 (N–H); 568 (M–O); 492 (M–N). ¹H-NMR spectrum (400 MHz, DMSO-d₆), δ , ppm: 11.71 (1H, s, N–NH), 10.91 (¹H, s, Ar–OH), 8.94 (1H, s, CH=N), 6.87–7.50 (5H, m, Ar–H), 2.27 (3H, s, Ar–CH₃). Elemental analysis found%: C 44.28; H 3.75; N 13.45; S 13.06. C₁₉H₁₉N₅S₂O₂ClNi. Calculated, %: C 43.46; H 3.26; N 13.34; S 12.21, UV-Vis (DMSO, $\epsilon \times 10^{-4}$ M), λ_{max} , nm: 383, 663.

2.2.2 H₁Cu. Yield 0.3010 g (61%), dark burgundy solid, mp 180 °C. IR spectrum (KBr), ν , cm⁻¹: 3345 (OH); 3078 (CH)_{aro.}; 1616 (CH=N); 1520 (CH=N)_{lyzi.}; 1475 (C=C); 1207, 815 (C=S); 738 (C–S–C); 1017 (N–N); 3247 (N–H); 559 (M–O); –(M–N). Elemental analysis found%: C 47.17; H 3.19; N 14.21; S 13.04. C₁₉H₁₆N₅S₂O₂ClCu. Calculated, %: C 46.24; H 3.27; N 14.19; S 12.99, UV-Vis (DMSO, $\epsilon \times 10^{-4}$ M), λ_{max} , nm: 441, 889.

2.2.3 H₂Ni. Yield 0.4120 g (72%), burgundy solid, mp 197 °C. IR spectrum (KBr), ν , cm⁻¹: 3337 (OH); 770 (H₂O); 3049 (CH)_{aro.}; 1603 (CH=N); 1520 (CH=N)_{lyzi.}; 1475 (C=C); 1210, 820 (C=S); 750 (C–S–C); 1014 (N–N); 3231 (N–H); 574 (M–O); 501 (M–N). ¹H-NMR spectrum (400 MHz, DMSO-d₆), δ , ppm: 11.46 (1H, s, N–NH), 10.45 (1H, s, Ar–OH), 8.18 (1H, s, CH=N), 6.95–7.69 (5H, m, Ar–H). Elemental analysis found%: C 38.01; H 2.64; N 12.27; S 11.17. C₁₈H₁₆N₅S₂O₂ClBrNi. Calculated, %: C 36.65; H 2.39; N 11.87; S 10.87, UV-Vis (DMSO, $\epsilon \times 10^{-4}$ M), λ_{max} , nm: 380, 660.

2.2.4 H₂Cu. Yield 0.3629 g (65%), dark burgundy solid, mp 185 °C. IR spectrum (KBr), ν , cm⁻¹: 3330 (OH); 3065 (CH)_{aro.}; 1615 (CH=N); 1506 (CH=N)_{lyzi.}; 1471 (C=C); 1212, 817 (C=S); 747 (C–S–C); 1011 (N–N); 3228 (N–H); 596 (M–O); 479 (M–N). Elemental analysis found%: C 38.42; H 2.22; N 12.49; S 11.37. C₁₈H₁₃N₅S₂O₂ClBrCu. Calculated, %: C 38.72; H 2.35; N 12.54; S 11.48, UV-Vis (DMSO, $\epsilon \times 10^{-4}$ M), λ_{max} , nm: 431, 887.

2.2.5 H₃Ni. Yield 0.3222 g (59%), burgundy solid, mp 201 °C. IR spectrum (KBr), ν , cm⁻¹: 3317 (OH); 781 (H₂O); 3080 (CH)_{aro.}; 1616 (CH=N); 1503 (CH=N)_{lyzi.}; 1456 (C=C); 1208, 821 (C=S); 751 (C–S–C); 1006 (N–N); 3219 (N–H); 562 (M–O); 490 (M–N). ¹H-NMR spectrum (400 MHz, DMSO-d₆), δ , ppm: 11.66 (1H, s, N–NH), 10.59 (1H, s, Ar–OH), 8.22 (1H, s, CH=N), 7.02–7.68 (5H, m, Ar–H), 2.27 (3H, s, Ar–CH₃). Elemental analysis found%: C 38.99; H 2.67; N 12.87; S 11.66. C₁₈H₁₅N₅S₂O₂FCl₂Ni. Calculated, %: C 38.37; H 2.33; N 12.43; S 11.38, UV-Vis (DMSO, $\epsilon \times 10^{-4}$ M), λ_{max} , nm: 379, 661.

2.2.6 H₃Cu. Yield 0.3191 g (60%), dark burgundy solid, mp 189 °C. IR spectrum (KBr), ν , cm⁻¹: 3321 (OH); 3078 (CH)_{aro.}; 1612 (CH=N); 1505 (CH=N)_{lyzi.}; 1453 (C=C); 1205, 825 (C=S); 751 (C–S–C); 1009 (N–N); 3223 (N–H); 561 (M–O); 488 (M–N). Elemental analysis found%: C 39.95; H 2.30; N 13.21; S 12.19. C₁₈H₁₂N₅S₂O₂FCl₂Cu. Calculated, %: C 40.65; H 2.27; N 13.17; S 12.06, UV-Vis (DMSO, $\epsilon \times 10^{-4}$ M), λ_{max} , nm: 428, 893.

2.2.7 H₄Ni. Yield 0.3312 g (63%), burgundy solid, mp 192 °C. IR spectrum (KBr), ν , cm⁻¹: 3331 (OH); 792 (H₂O); 3063 (CH)_{aro.}; 1621 (CH=N); 1501 (CH=N)_{lyzi.}; 1456 (C=C); 1203, 842



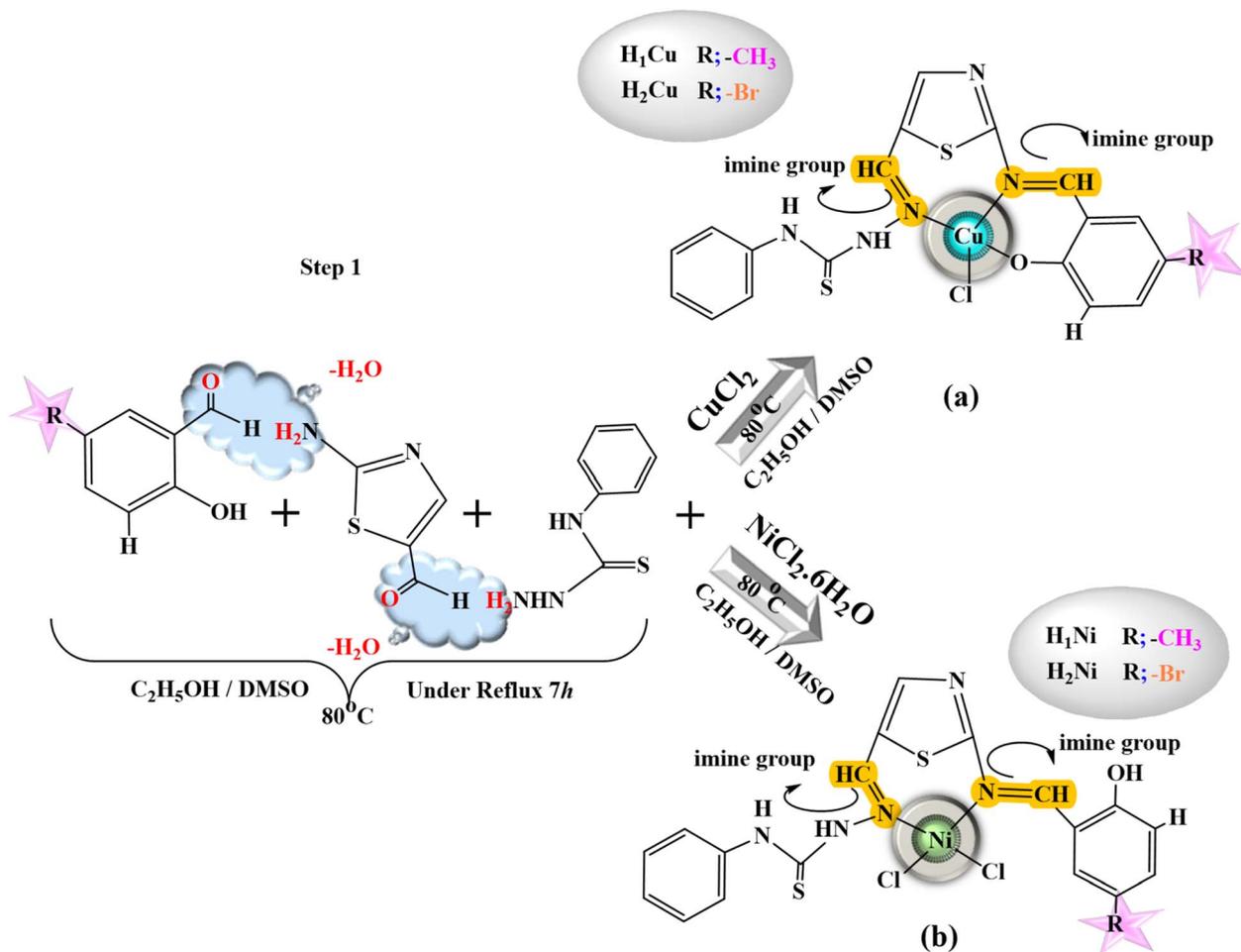


Fig. 1 General procedure for the heterocyclic thiosemicarbazone (a) Cu complexes (H_1Cu , H_2Cu) and (b) Ni complexes (H_1Ni , H_2Ni).

(C=S); 749 (C-S-C); 1005 (N-N); 3244 (N-H); 570 (M-O); 485 (M-N). 1H -NMR spectrum (400 MHz, DMSO- d_6), δ , ppm: 11.25 (1H, s, N-NH), 10.61 (1H, s, Ar-OH), 8.29 (1H, s, CH=N), 7.11–7.82 (5H, m, Ar-H). Elemental analysis found%: C 43.55; H 3.71; N 13.51; S 12.49. $C_{19}H_{18}N_5S_2O_2FCu$. Calculated, %: C 42.02; H 2.97; N 12.90; S 11.81, UV-Vis (DMSO, $\epsilon \times 10^{-4}$ M), λ_{max} , nm: 377, 659.

2.2.8 H_4Cu . Yield 0.2813 g (55%), dark burgundy solid, mp $187^\circ C$. IR spectrum (KBr), ν , cm^{-1} : 3325 (OH); 3078 (CH)_{aro}; 1616 (CH=N); 1503 (CH=N)_{lyz}; 1456 (C=C); 1208, 842 (C=S); 753 (C-S-C); 1015 (N-N); 3241 (N-H); 575 (M-O); 504 (M-N). Elemental analysis found%: C 44.79; H 2.99; N 13.21; S 13.01. $C_{19}H_{15}N_5S_2O_2FCu$. Calculated, %: C 44.62; H 2.96; N 13.69; S 12.54, UV-Vis (DMSO, $\epsilon \times 10^{-4}$ M), λ_{max} , nm: 429, 891.

2.3. Antimicrobial assay

Gram (+) bacteria, Gram (-) bacteria and yeast used in the antimicrobial study are as follows, respectively: (*Micrococcus luteus* ATCC9341, *Staphylococcus epidermidis* ATCC12228, *Bacillus cereus* RSKK863), (*Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumonia* ATCC27853, *Enterobacter aerogenes* ATCC51342, *Salmonella typhi* H NCTC9018394, *Shigella*

dysenteria NCTC2966, *Proteus vulgaris* RSKK96026) and (*Candida albicans* Y-1200-NIH). For the antimicrobial assay, all heterocyclic thiosemicarbazone complexes (H_1Ni , H_2Ni , H_3Ni , H_4Ni , H_1Cu , H_2Cu , H_3Cu , and H_4Cu) were screened against these disease agent pathogens by the well-diffusion methods.^{29–31} In this method, it was determined that DMSO, used as solvent control, did not show antimicrobial activity against the tested organisms. As a first step, all heterocyclic thiosemicarbazone complexes were solved ($3.5 \mu g mL^{-1}$) in DMSO, and all pathogenic microorganisms were incubated in Nutrient Broth agar (10^6 CFU mL^{-1}) for 24 h at $37^\circ C$. As a second step, these cultures were then homogenized by adding them to Mueller–Hinton Agar (MHA) cooled to $45^\circ C$, and they were poured into sterile Petri dishes and cooled. Afterward, wells of 6 mm diameter were pierced in these agars, and the heterocyclic thiosemicarbazone complexes were added. Finally, the plates were incubated in an oven (at $37^\circ C$, 24 h), the inhibition zone of all heterocyclic compounds was measured, and then the average of the activity values performed with two repetitions was taken. As a third second, selected disease agent pathogens were compared with standard antibiotics. For this purpose, Ampicillin (AMP10), Sulphamethoxazole (SXT25),



Table 1 Some physical properties, analytical and organic elemental analysis data of heterocyclic thiosemicarbazone complexes^a

Symbol	Chemical formula (M_w , g mol ⁻¹)	Colour conductance ($\mu\text{S cm}^{-1}$)	M. p. (°C)	Elemental analysis calculated (found)%			
			μ_{eff} (BM)	C	H	N	S
H ₁ Ni	C ₁₉ H ₁₇ N ₅ S ₂ OCl ₂ Ni (525.09)	Burgundy 35.6	192 D	44.95 (43.46)	3.77 (3.26)	13.80 (13.34)	12.63 (12.21)
H ₁ Cu	C ₁₉ H ₁₆ N ₅ S ₂ OClCu (493.49)	Dark burgundy 15.4	180 1.93	46.24 (47.17)	3.27 (3.19)	14.19 (14.21)	12.99 (13.04)
H ₂ Ni	C ₁₈ H ₁₄ N ₅ S ₂ OCl ₂ BrNi (586.86)	Burgundy 2.5	197 D	37.76 (36.65)	2.82 (2.39)	12.23 (11.87)	11.20 (10.87)
H ₂ Cu	C ₁₈ H ₁₃ N ₅ S ₂ OClBrCu (558.36)	Dark burgundy 15.6	185 1.81	38.72 (38.42)	2.35 (2.22)	12.54 (12.49)	11.48 (11.37)
H ₃ Ni	C ₁₈ H ₁₃ N ₅ S ₂ OFC ₃ Ni (563.50)	Burgundy 21.8	201 D	39.59 (38.37)	2.77 (2.33)	12.83 (12.43)	11.74 (11.38)
H ₃ Cu	C ₁₈ H ₁₂ N ₅ S ₂ OFC ₂ Cu (531.89)	Dark burgundy 17.0	189 2.11	40.65 (39.95)	2.27 (2.30)	13.17 (13.21)	12.06 (12.19)
H ₄ Ni	C ₁₉ H ₁₆ N ₅ S ₂ OFC ₂ Ni (543.48)	Burgundy 9.0	192 D	43.41 (42.02)	3.45 (2.97)	13.32 (12.90)	12.20 (11.81)
H ₄ Cu	C ₁₉ H ₁₅ N ₅ S ₂ OFC ₁ Cu (511.48)	Dark burgundy 55.7	187 1.98	44.62 (44.79)	2.96 (2.99)	13.69 (13.21)	12.54 (13.01)

^a D: diamagnetic.

UV-Vis spectra data of all heterocyclic thiosemicarbazone Ni²⁺ and Cu²⁺ complexes are presented in Table 4. The electronic spectra of all nickel and copper complexes showed two main bands. $\pi \rightarrow \pi^*$ transitions of the aromatic ring and $n \rightarrow \pi^*$ transitions of the imine group were observed in the ranges of 231–252 nm and 323–327 nm, respectively. In the electronic spectra of nickel complexes, the absorption bands that appeared in the 377–383 nm range were assigned to metal-ligand charge transfer transitions. The bands observed in the 659–663 nm range were assigned to the d-d transitions, which is compatible with the Ni²⁺ square planar geometry.³⁴ In the electronic spectra of copper complexes, the absorption bands observed in the 428–441 nm range were assigned to intra-ligand charge transfer transitions.³⁵ The bands that appeared in the 887–893 nm range were assigned to the d-d transitions, which is compatible with the Cu(II) tetrahedral geometry.³⁶

Table 3 ¹H-NMR chemical shift (ppm) of heterocyclic thiosemicarbazone Ni(II) complexes

Compound	N-NH	Ar-OH	CH=N	Ar-H	Ar-CH ₃
H ₁ Ni	11.71	10.91	8.94	6.87–7.50	2.27
H ₂ Ni	11.46	10.45	8.18	6.95–7.69	—
H ₃ Ni	10.66	10.59	8.22	7.02–7.68	2.27
H ₄ Ni	11.25	10.61	8.29	7.11–7.82	—

The magnetic moments of heterocyclic thiosemicarbazone Ni²⁺ complexes were determined diamagnetic, confirming the existence of square planar geometry.³⁷ The magnetic moments of heterocyclic thiosemicarbazone Cu²⁺ complexes were observed in the range of 1.81–2.11 B.M., indicating the presence

Table 2 FT-IR spectra data of heterocyclic thiosemicarbazone complexes

Symbol	$\nu(\text{OH})$	$\nu(\text{CH})_{\text{aro.}}/\nu(\text{C}=\text{C})$	$\nu(\text{CH}=\text{N})/\nu(\text{CH}=\text{N})_{\text{vib}}$	$\nu(\text{C}=\text{S})$	$\nu(\text{C}-\text{S}-\text{C})$	$\nu(\text{N}-\text{N})/\nu(\text{N}-\text{H})$	$\nu(\text{M}-\text{O})/\nu(\text{M}-\text{N})$
H ₁ Ni	3340	3025 1495	1615 1544	1210 819	750	1003 3243	— 492
H ₁ Cu	—	3078 1475	1616 1520	1207 815	738	1017 3247	559 490
H ₂ Ni	3337	3049 1475	1603 1520	1210 820	750	1014 3231	— 501
H ₂ Cu	—	3065 1471	1615 1506	1212 817	747	1011 3228	596 479
H ₃ Ni	3317	3080 1456	1616 1503	1208 821	751	1006 3219	— 490
H ₃ Cu	—	3078 1453	1612 1505	1205 825	751	1009 3223	561 488
H ₄ Ni	3331	3063 1456	1621 1501	1203 842	749	1005 3244	— 485
H ₄ Cu	—	3078 1456	1616 1503	1208 842	753	1015 3241	575 504



Table 4 TGA and UV-Vis data of heterocyclic thiosemicarbazone complexes

Compound	Charge transfer transition (nm)	d-d transition (nm)	Step	T_1 (°C)	T_f (°C)	Residue mass at 800 °C (wt%)
H ₁ Ni	383	663	1st	115.02	261.31	3.46
			2nd	320.89	408.36	
			3rd	437.02	624.15	
H ₁ Cu	441	889	1st	106.89	166.67	3.41
			2nd	223.70	386.71	
			3rd	471.22	644.75	
H ₂ Ni	380	660	1st	110.14	208.30	6.05
			2nd	214.18	391.47	
			3rd	488.93	670.19	
H ₂ Cu	431	887	1st	121.79	387.13	5.32
			2nd	494.24	678.22	
H ₃ Ni	379	661	1st	158.26	342.89	6.53
			2nd	410.75	709.38	
H ₃ Cu	428	893	1st	102.86	203.77	3.84
			2nd	253.80	422.66	
			3rd	482.08	691.43	
H ₄ Ni	377	659	1st	111.95	351.47	2.12
			2nd	394.44	620.19	
H ₄ Cu	429	891	1st	101.57	200.36	12.17
			2nd	267.10	462.03	
			3rd	490.31	544.63	
			4th	553.66	687.51	

of one unpaired electron in the Cu²⁺ ion.³⁸ The paramagnetic behavior confirms the existence of tetrahedral geometry.³⁹

The molar conductance of heterocyclic thiosemicarbazone Ni²⁺ and Cu²⁺ complexes was determined in the range 2.5–35.6 μS cm⁻¹ and 15.4–55.7 μS cm⁻¹, respectively, confirming the non-electrolyte nature of all complexes in DMSO solution.⁴⁰

The graphical illustration and the photographs of inhibition regions of pathogenic bacterial species are presented in Fig. 3–5, respectively. The heterocyclic thiosemicarbazone Ni²⁺ and Cu²⁺ complexes were tested *in vitro* against selected disease-causing pathogenic bacteria and yeast to determine their antibacterial and antifungal activities. The yeast and pathogens

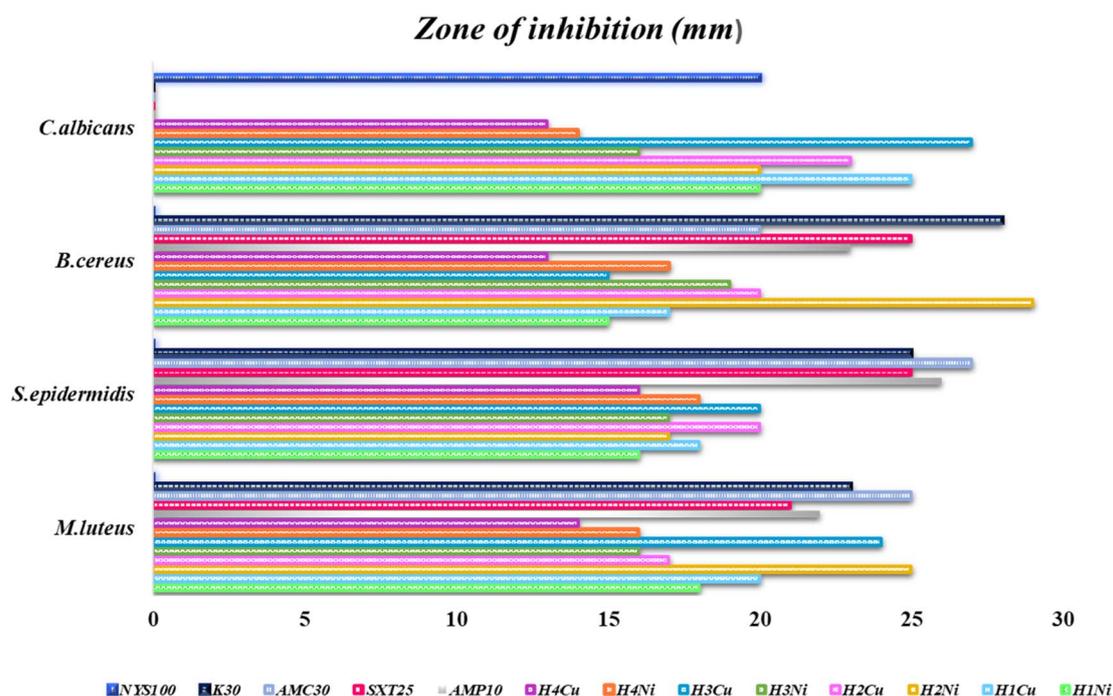


Fig. 3 Graphical illustration of Gram (+) pathogenic bacterial species, and standard antibiotics.



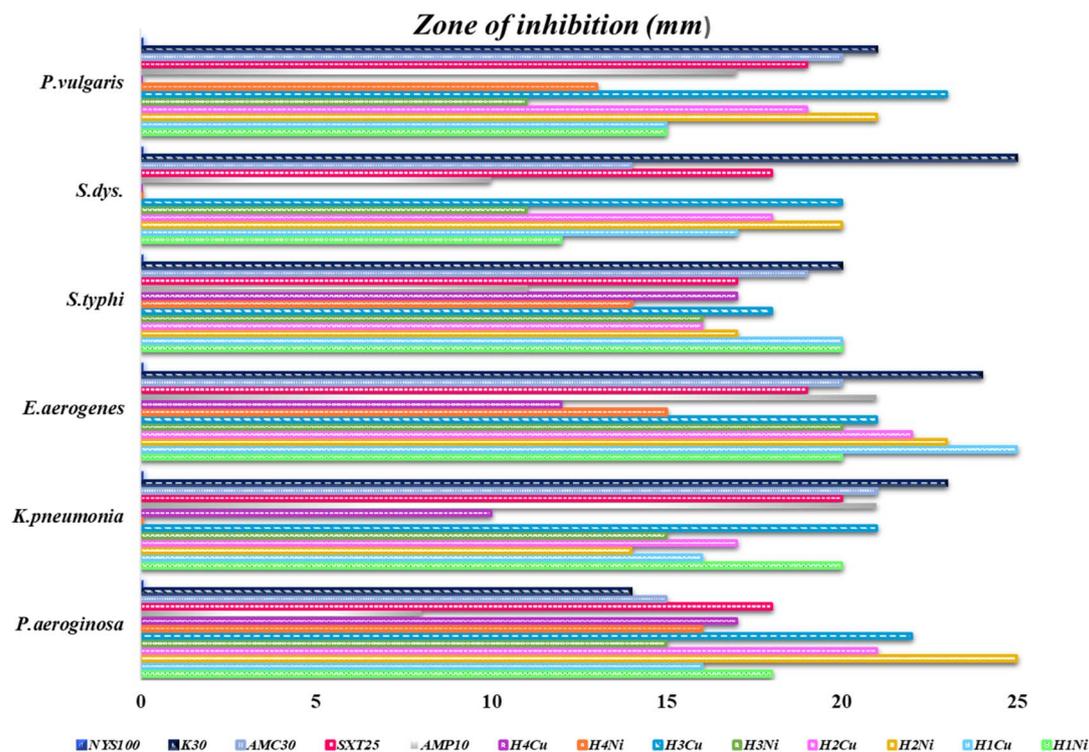


Fig. 4 Graphical illustration of Gram (-) pathogenic bacterial species, and standard antibiotics.

were compared to the standard antibiotics and anticandidal. The results showed that the compounds had different antimicrobial effects against Gram-positive bacteria (*M. luteus*, *S. epidermidis*, *B. cereus*), Gram-negative bacteria (*P. aeruginosa*, *K. pneumonia*, *E. aerogenes*, *S. typhi* H, *S. dysenteriae*, *P. vulgaris*) and yeast (*C. albicans*). The heterocyclic thiosemicarbazone Ni²⁺ and Cu²⁺ complexes generally exhibited more potent antibacterial capacity against Gram (-) than Gram (+) bacteria. H₂Ni (29 mm) complex showed more higher inhibitory effects than all standard antibiotics against the Gram (+) bacteria *B. cereus*. This pathogen causes certain infections such as vomiting and eye.⁴¹ H₃Cu and H₂Ni complexes (24 mm and 25 mm, respectively) demonstrated higher activity than the standard antibiotics (except AMC30) against the Gram (+) bacteria *M. luteus*. This pathogen causes bloodstream infections.⁴² Among Gram (-) bacteria, all heterocyclic thiosemicarbazone complexes (16–25 mm) showed significant antibacterial activities against *P. aeruginosa* and *S. typhi* H compared to the standard antibiotics. *S. typhi* H bacteria causes typhoid.⁴³ H₂Ni (25 mm), H₂Cu (21 mm), H₃Cu (22 mm) complexes showed a more potent inhibitory impact than all standard antibiotics against *P. aeruginosa*. This bacteria causes urinary tract infections.⁴⁴ H₃Cu (23 mm) demonstrated a potent antibacterial activity than all standard antibiotics against *P. vulgaris*. This pathogen causes certain infections, such as meningitis, diarrhea, and abscesses.⁴⁵ H₁Cu (25 mm) showed more higher activity than all standard antibiotics against *E. aerogenes*. This pathogen causes infections in the urinary and respiratory tracts.⁴⁶ Additionally, the antifungal effects of the heterocyclic thiosemicarbazone Ni²⁺ and Cu²⁺

complexes were compared to the standard Nystatin antibiotic against yeast *C. albicans*. This fungus causes gastrointestinal tract infections and bloodstream infections.⁴⁷ Cu²⁺ complexes generally exhibited a more potent effect than Ni²⁺ complexes. H₃Cu (27 mm), H₁Cu (25 mm), and H₂Cu (23 mm) complexes showed much stronger antifungal activity than Nystatin. H₁Ni (20 mm) and H₂Ni (20 mm) complexes demonstrated as much inhibitory impact as standard antibiotics.

In conclusion, the heterocyclic thiosemicarbazone Ni²⁺ and Cu²⁺ complexes were determined to have high or moderate antifungal and antibacterial activity. It was observed that the antibacterial and antifungal activities of thiosemicarbazone compounds, including electron-withdrawing groups (-Br/-Cl/-F), were more effective than the thiosemicarbazone compounds, including electron-donating group (-CH₃). The synthesized thiosemicarbazone compounds showed very good antibacterial and antifungal properties because they contain thiazole rings containing N and S heteroatoms, nickel and copper metal ions with chelation ability, and asymmetric diimine groups that impart biological activity. This situation is consistent with similar research results.^{24,48,49} The antimicrobial activities vary depending on the presence of metal ions, the position of the substituent on the ring, and the presence of terminal groups (-CH₃, -Ph, -F, -Cl, -Br, etc.). Increased and/or decreased biological activities have been reported for compounds.²⁷ The antimicrobial activities increase and/or decrease depending on the presence of metal ions, the position of the substituent on the ring, and the presence of terminal groups (-CH₃, -Ph, -F, -Cl, -Br, etc.).



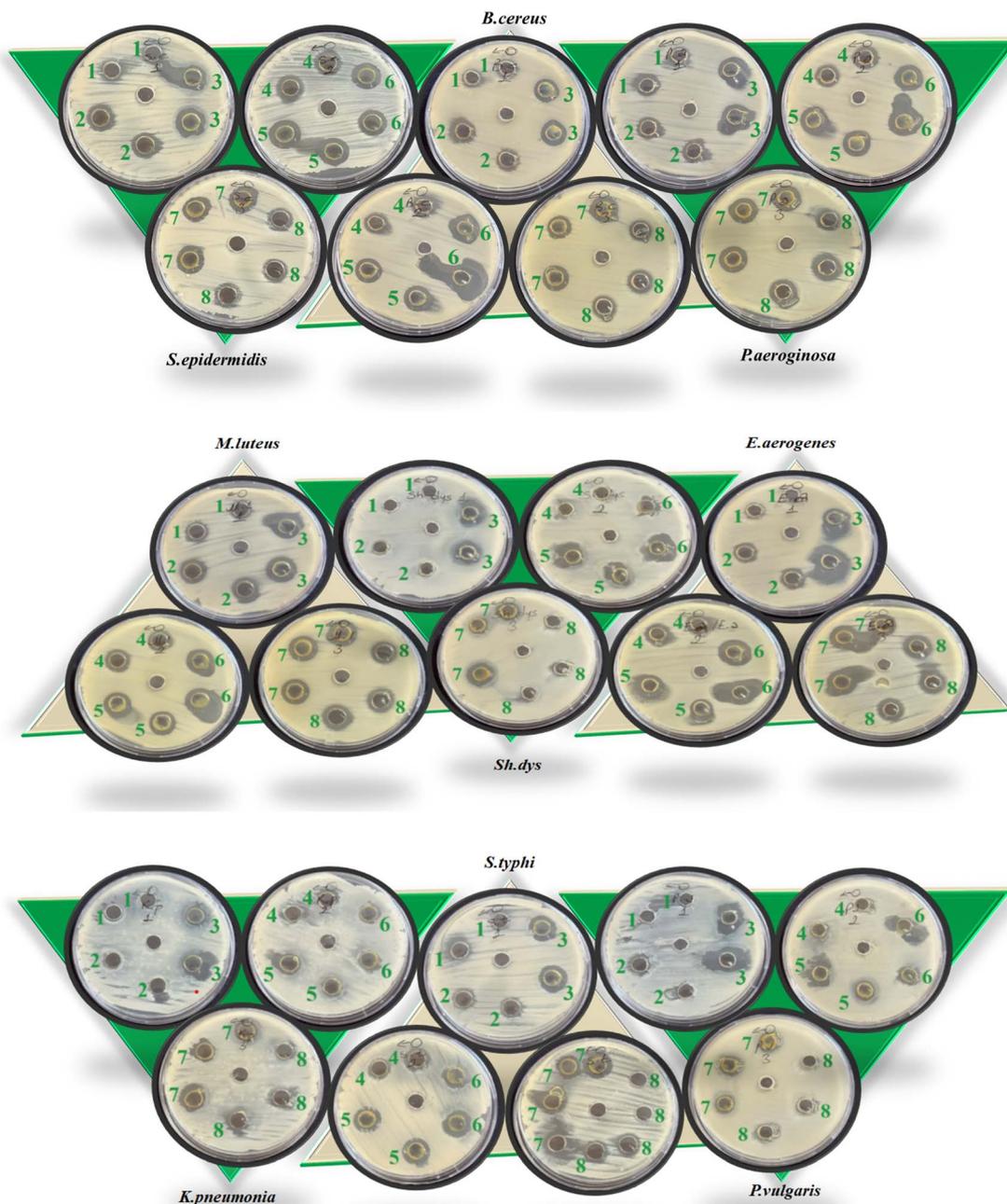


Fig. 5 Photographs of inhibition zones (mm) of some Gram (+) and Gram (-) bacteria.

Ni^{2+} complexes generally showed stronger antibacterial activity than Cu^{2+} complexes. Especially the H_2Ni complex exhibited a higher inhibitory effect.

4 Conclusions

In this study, novel α -N-heterocyclic thiosemicarbazone compounds were synthesized using the template method and were characterized by different spectroscopic techniques. Based on the spectral studies, it was determined that Ni^{2+} complexes had a square planar geometry, while Cu^{2+} complexes had

tetrahedral geometry. The biological activities of heterocyclic thiosemicarbazone compounds were evaluated against disease-causing pathogens using the well-diffusion method. All heterocyclic thiosemicarbazone complexes were observed to have high or moderate antimicrobial activity. The synthesized thiosemicarbazone compounds containing asymmetric azomethine groups exhibited different biological activities depending on the presence of metal ions and the presence of terminal groups. The heterocyclic thiosemicarbazone Ni^{2+} and Cu^{2+} complexes exhibited more potent impacts against different Gram-negative bacterial strains as potential antibacterial



agents. The heterocyclic thiosemicarbazone Ni²⁺ complexes showed more potent impacts against yeast as potential anti-fungal agents. According to high/or moderate biological activity results, the synthesized novel α -N-heterocyclic thiosemicarbazone compounds can be recommended as potent inhibitors in diverse pharmaceutical, biological, medicinal, biomedical, *etc.* applications.

Data availability

The data supporting this article have been included in the main article and the ESI.†

Author contributions

D. Nartop designed the experiments and contributed to the interpretation of the results. E. Hasanoglu Özkan carried out the experiments depend on synthesis. D. Nartop and E. Hasanoglu Özkan performed visualization, formal analysis, writing – original draft, writing-review, and editing, H. Ogutcu carried out the antimicrobial experiments and assessments. N. Kurnaz Yetim and İ. Özdemir contributed to conceptualization and review. Each author contributed to the final manuscript and discussed the findings.

Conflicts of interest

There are no conflicts to declare between the authors.

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References

- D. Bandyopadhyay and B. K. Banik, *Microwave-induced Synthesis of Heterocycles of Medicinal Interests: In Green Synthetic Approaches for Biologically Relevant Heterocycles*, Elsevier Inc., 2015, vol. 1, pp. 517–557.
- V. J. Ram, A. Sethi, M. Nath and R. Pratap, *The Chemistry of Heterocycles: Nomenclature and Chemistry of Three to Five Membered Heterocycles*, Elsevier, 2019.
- P. A. Taylor, R. P. Robinson, Y. M. Fobian, D. C. Blakemore, L. H. Jones and O. Fadeyi, Modern advances in heterocyclic chemistry in drug discovery, *Org. Biomol. Chem.*, 2016, **14**(28), 6611–6637.
- G. C. dos Santos, L. M. Martins, B. A. Bregadiolli, V. F. Moreno, L. C. da Silva-Filho and B. H. S. T. da Silva, Heterocyclic compounds as antiviral drugs: synthesis, structure-activity relationship and traditional applications, *J. Heterocycl. Chem.*, 2021, **58**(427), 1–35.
- S. P. Palaniappan, Pharmacological role of heterocyclic compounds in the treatment of Alzheimer's disease: A Review, *J. Phytopharm.*, 2022, **11**(4), 289–294.
- S. Pearce, The importance of heterocyclic compounds in anti-cancer drug design, *Drug Discovery World*, 2017, **18**(2), 66–70.
- S. Argibay-Otero, R. Carballo and E. M. Vázquez-López, Coordination chemistry of potentially S,N,Npy-Tridentate thiosemicarbazones with the {Re(CO)₃}⁺ fragment and formation of hemiaminal derivatives, *Inorg. Chem.*, 2023, **62**(1), 224–237.
- E. B. Seená, M. Sithambaresan, S. Vasudevan, *et al.*, Structural and spectral characterization of Cu(II) complexes of N(4)-substituted thiosemicarbazones derived from 2-hydroxyacetophenone: Crystal structure of a dinuclear Cu(II) complex, *J. Chem. Sci.*, 2020, **132**(149), 1–11.
- A. I. Matesanz and P. Souza, Unprecedented Pt(II) complex of an asymmetric 2,6-diacetylpyridine bis(4N-substituted thiosemicarbazone) ligand, *Inorg. Chem. Commun.*, 2013, **27**, 5–8.
- F. Muleta and T. A. R. Eswaramoorthy, A Review on synthesis, characterization methods and biological activities of semicarbazone, thiosemi-carbazone and their transition metal complexes, *J. Nat. Sci. Res.*, 2019, **9**(17), 33–46.
- N. Farrell, Biomedical uses and applications of inorganic chemistry, *Coord. Chem. Rev.*, 2002, **232**(1–2), 1–4.
- D. West, S. Padhye and P. Sonawane, in *Structure and Bonding*, Springer-Verlag, New York, 1991, vol. 76, pp. 1–49.
- F. Basuli, S. M. Peng and S. Bhattacharya, Unusual coordination mode of thiosemicarbazone ligands. a search for the origin, *Inorg. Chem.*, 2000, **39**(6), 1120–1127.
- S. Fernández-Fariña, I. Velo-Helena, M. Martínez-Calvo, A. M. González-Noya and R. Pedrido, Design, synthesis and structural characterization of a novel asymmetric hydrazone-thiosemicarbazone ligand with the aim of obtaining interesting metallosupramolecular architectures, *ChemistrySelect*, 2022, **8**(16), 1–5.
- D. U. Kirtani, N. S. Ghatpande, K. R. Suryavanshi, P. P. Kulkarni and A. A. Kumbhar, Fluorescent copper(II) complexes of asymmetric bis(thiosemicarbazone)s: Electrochemistry, cellular uptake and antiproliferative activity, *ChemistrySelect*, 2021, **6**, 6063–6070.
- N. Özdemir, M. Şahin, T. Bal-Demirci and B. Ülküseven, The asymmetric ONNO complexes of dioxouranium(VI) with N1,N4-diarylidene-S-propyl-thiosemicarbazones derived from 3,5-dichlorosalicylaldehyde: Synthesis, spectroscopic and structural studies, *Polyhedron*, 2011, **30**(3), 515–521.
- T. Varadinova, D. Kovala-Demertzi, M. Rupelieva, M. Demertzis and P. Genova, Antiviral activity of platinum (II) and palladium (II) complexes of pyridine-2-carbaldehyde thiosemicarbazone, *Acta Virol.*, 2001, **45**(2), 87–94.
- S. N. Pandeya and J. R. Dimmock, Recent evaluations of thiosemicarbazones and semicarbazones and related compounds for antineoplastic and anticonvulsant activities, *Pharmazie*, 1993, **48**(9), 659–666.
- N. C. Kasuga, K. Sekino, C. Kuomo, N. Shimada, M. Ishikawa and K. Nomiya, Synthesis, structural characterization and antimicrobial activities of 4- and 6-coordinate nickel (II)



- complexes with three thiosemicarbazones and semicarbazone ligands, *J. Inorg. Biochem.*, 2001, **84**, 55–65.
- 20 R. Sharma, S. K. Agarwal, S. Rawat and M. Nagar, Synthesis, characterization and antibacterial activity of some transition metal cis 3,7-dimethyl-2,6 octadienemicarbazone complexes, *Transition Met. Chem.*, 2006, **31**(2), 201–206.
- 21 H. Alshater, A. I. Al-Sulami, S. A. Aly, E. M. Abdalla, M. A. Sakr and S. S. Hassan, Antitumor and antibacterial activity of Ni(II), Cu(II), Ag(I), and Hg(II) complexes with ligand derived from thiosemicarbazones: Characterization and theoretical studies, *Molecules*, 2023, **28**(2590), 1–18.
- 22 S. Datta, D. K. Seth, S. Gangopadhyay, P. Karmakar and S. Bhattacharya, Nickel complexes of some thiosemicarbazones: Synthesis, structure, catalytic properties and cytotoxicity studies, *Inorg. Chim. Acta*, 2012, **392**, 118–130.
- 23 S. S. Renade and V. K. Panday, Transition metals in human cancer II, *Sci. Total Environ.*, 1984, **40**, 245–257.
- 24 D. Polo-Cerón, Cu(II) and Ni(II) complexes with new tridentate NNS thiosemicarbazones: synthesis, characterisation, DNA interaction, and antibacterial activity, *Bioinorg. Chem. Appl.*, 2019, **2019**, 1–14.
- 25 H. Karlsson, M. Fryknäs, S. Strese, J. Gullbo, G. Westman, U. Bremberg, T. Sjöblom, T. Pandzic, R. Larsson and P. Nygren, Mechanistic characterization of a copper containing thiosemicarbazone with potent antitumor activity, *Oncotarget*, 2017, **8**(18), 30217–30234.
- 26 J. J. R. Fraústo da Silva and R. P. Williams, The biological chemistry of the elements, *Chap. 15: The Inorganic Chemistry of Life*, *Oxónio*, Clarendon Press, 2nd edn, 1991.
- 27 S. Shivhare and M. D. Gautam, Electrochemical study of complexes of Cu(II) and Ni(II) with thiosemicarbazone, *J. Chem. Pharm. Res.*, 2011, **3**(6), 1009–1016.
- 28 A. Bult, *Metal Ions in Biological Systems*, ed. Sigel, H., Marcel Dekker, New York, 1983, vol. 16, pp. 261–263.
- 29 E. Ülke, E. Hasanoğlu Özkan, D. Nartop and H. Ogutcu, New antimicrobial polymeric microspheres containing azomethine, *J. Inorg. Organomet. Polym.*, 2022, **32**, 3971–3982.
- 30 T. Amutha, M. Rameshbabu, S. Sasi Florence, N. Senthilkumar, I. Vetha Potheher and K. Prabha, Studies on structural and optical properties of pure and transition metals (Ni, Fe and co-doped Ni-Fe) doped tin oxide (SnO₂) nanoparticles for anti-microbial activity, *Res. Chem. Intermed.*, 2019, **45**, 1929–1941.
- 31 D. Nartop, E. Hasanoğlu Özkan, H. Ögütçü and N. Kurnaz Yetim, Synthesis and in vitro biological assessments of novel thiazole-based thiosemicarbazone complexes, *Duzce University Journal of Science and Technology*, 2024, **12**(3), 1389–1397.
- 32 H. Alshater, A. I. Al-Sulami, S. A. Aly, E. M. Abdalla, M. A. Sakr and S. S. Hassan, Antitumor and antibacterial activity of Ni(II), Cu(II), Ag(I), and Hg(II) complexes with ligand derived from thiosemicarbazones: Characterization and theoretical studies, *Molecules*, 2023, **28**(6), 1–18.
- 33 R. M. Silverstein and F. X. Webster, *Spectrometric Identification of Organic Compounds*, John Wiley & Sons, New York, USA, 6th edn, 1998, vol. 11, pp. 160–166.
- 34 V. K. Maurya, L. B. Prasad, A. Singh, K. Shiv and A. Prasad, Synthesis, spectroscopic characterization, biological activity, and conducting properties of functionalized Ni(II) dithiocarbamate complexes with solvent extraction studies of the ligands, *J. Sulfur Chem.*, 2022, **44**(3), 336–353.
- 35 M. Hazra, T. Dolai, A. Pandey, S. K. Dey and A. Patra, Synthesis and characterisation of Copper(II) complexes with tridentate NNO functionalized ligand: density function theory study, DNA binding mechanism, optical properties, and biological application, *Bioinorg. Chem. Appl.*, 2014, **2014**, 1–14.
- 36 A. C. Tella and J. A. Obaleye, Copper(II) complexes of 4, 4-Diaminodiphenylsulphone: Synthesis, characterization and biological studies, *E-J. Chem.*, 2009, **6**(S1), S311–S323.
- 37 B. Atasever Arslan, B. Kaya, O. Şahin, S. Baday, C. C. Saylan and B. Ülküseven, The iron(III) and Nickel(II) complexes with tetradentate thiosemicarbazones: Synthesis, experimental, theoretical characterization, and antiviral effect against SARS-CoV-2, *J. Mol. Struct.*, 2021, **1246**(131166), 1–7.
- 38 L. V. Gavali, A. A. Mohammed, M. J. K. Al-Ogaili, S. H. Gaikwad, M. Kulkarni, R. Das and P. A. Ubale, Novel terephthalaldehyde bis(thiosemicarbazone) Schiff base ligand and its transition metal complexes as antibacterial Agents: Synthesis, characterization and biological investigations, *Results Chem.*, 2024, **7**(101316), 1–9.
- 39 M. V. Angelusiu, S. F. Barbuceanu, C. Draghici and G. L. Almajan, New Cu(II), Co(II), Ni(II) complexes with aroyl-hydrazone based ligand: Synthesis, spectroscopic characterization and in vitro antibacterial evaluation, *Eur. J. Med. Chem.*, 2010, **45**(5), 2055–2062.
- 40 E. Hasanoğlu Özkan and N. Sari, Use of immobilized novel dendritic molecules as a marker for the detection of glucose in artificial urine, *J. Mol. Struct.*, 2020, **1201**(127134), 1–7.
- 41 A. Z. Macit, E. Hasanoğlu Özkan, H. Ogutcu and D. Nartop, Synthesis and in vitro antimicrobial evaluation of novel potent bioactive heterocyclic compounds, *Polycyclic Aromat. Compd.*, 2023, 1–12.
- 42 S. Kocoglu, Z. Hayvalı and H. Ogutcu, A Polydentate ligand based on 2,2'-Dipyridylamine unit linked benzo-15-Crown-5; alkali and transition metal complexes; Photoresponsive ligand; antimicrobial evaluation against pathogenic microorganisms, *Transition Met. Chem.*, 2021, **46**(7), 509–522.
- 43 M. Ulular, N. Sari, F. Han, H. Ögütçü and E. Hasanoğlu Özkan, Synthesis, antimicrobial, and DNA-binding evaluation of novel schiff bases containing tetrazole moiety and their Ni(II) and Pt(II) Complexes, *Pharm. Chem. J.*, 2024, **57**(10), 1609–1620.
- 44 D. Nartop, E. Tokmak, E. Hasanoğlu Özkan, H. E. Kızıl, H. Ögütçü, G. Ağar and S. Allı, Synthesis of novel polymers containing Schiff base as potential antimutagenic and antimicrobial agents, *J. Med. Chem. Sci.*, 2020, **4**(3), 363–372.



- 45 D. Drzewiecka, Significance and roles of *Proteus* spp. bacteria in natural environments, *Microb. Ecol.*, 2016, **72**(4), 741–758.
- 46 J. P. S. Cabral, Water microbiology. Bacterial pathogens and water, *Int. J. Environ. Res. Public Health*, 2010, **7**(10), 3657–3703.
- 47 H. Carolus, K. V. Dyck and P. V. Dijck, *Candida albicans* and *Staphylococcus* species: A threatening twosome, *Front. Microbiol.*, 2019, **10**(2162), 1–8.
- 48 V. A. Kumar, Y. Sarala, A. Siddikha, S. Vanitha, S. Babu and A. V. Reddy, Synthesis, characterization antimicrobial and antioxidant activities of 2,4-dihydroxybenzaldehyde-4-phenyl-3-thiosemicarbazone (DHBPTSC) and its Pd(II), Ni(II)dppm mixed ligand and Cu(II) complex having heterocyclic bases, *J. Appl. Pharm. Sci.*, 2018, **8**(4), 071–078.
- 49 P. Jain, S. Sharma, N. Kumar and N. Misra, Ni(II) and Cu(II) complexes of bidentate thiosemicarbazone ligand: Synthesis, structural, theoretical, biological studies and molecular modeling, *Appl. Organomet. Chem.*, 2020, **34**, 9.

