



Original Research Article

Synthesis, Chemical and Biological Activity Studies of Azo-Schiff Base Ligand and Its Metal Complexes

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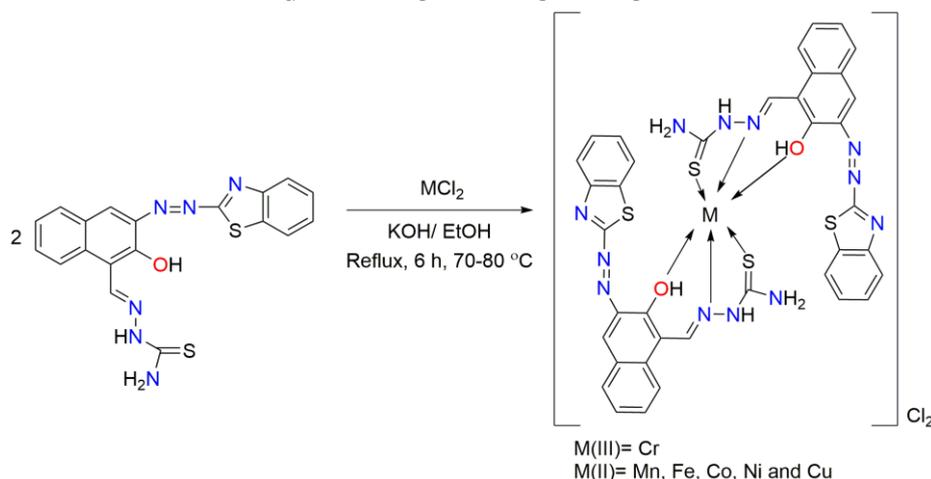
2-Hydroxy-1-naphthlaldehyde Metal complexes

Thiosemicarbazides

ABSTRACT

A new Azo-Schiff base ligand was prepared by reaction of 2-aminobenzothiazole and 2-hydroxy-1-naphthlaldehyde to form the azo compound. In the latest step, the azo compound was reacted with thiosemicarbazide to give the ligand. The synthesized ligand was used for complexes with different metal ions like Ni(II), Co(II), Cr(III), Mn(II), and Cu(II) by using a molar ratio of ligand: metal as 1:2. Resulted in compounds were characterized by FT-IR, ¹HNMR, ¹³CNMR, UV-Vis spectroscopy, Mass, elemental analysis, magnetic moment and molar conductivity studies. The ligand and its complexes' activity were tested against four types of bacteria and one type of fungi; some compounds gave a positive result, and others showed negative inhibition.

GRAPHICAL ABSTRACT



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Introduction

The presence of the azo moiety distinguishes the azo compounds or dyes ($-N=N-$) in their structure, conjugated with two distinct or identical mono- or polycyclic aromatic or hetero-aromatic systems [1]. Azo dyes are synthesized *via* two steps reaction, the first being the synthesis of an aromatic diazonium ion from an aniline derivative. The second step is coupling the diazonium salt with an aromatic compound [2]. The azo groups generally connected to benzene and naphthalene rings but can also be attached to aromatic heterocycles or enolizable aliphatic groups [3]. Azo dyes acquired a broad interest in application to a biological system and as indicators in complex colorimetric titration in analytical chemistry [4]. The Schiff base reaction refers to the interaction between compounds having amino groups (NH_2 , NH_2OH , etc.) and carbonyl groups (aldehydes or ketones); Schiff bases are compounds that include an azo methine group ($-CH=N-$) [5]. In 2021, a new azo-Schiff base ligand was prepared by coupling reaction of the diazonium salt with an appropriate amount of (imidazole derivative) as a coupling component in an alkaline solution [6]. The study aimed to synthesize and characterize new azo-Schiff compounds and its metal complexes, then test the ligand and complexes' efficient biological activity against various types of bacteria and fungi.

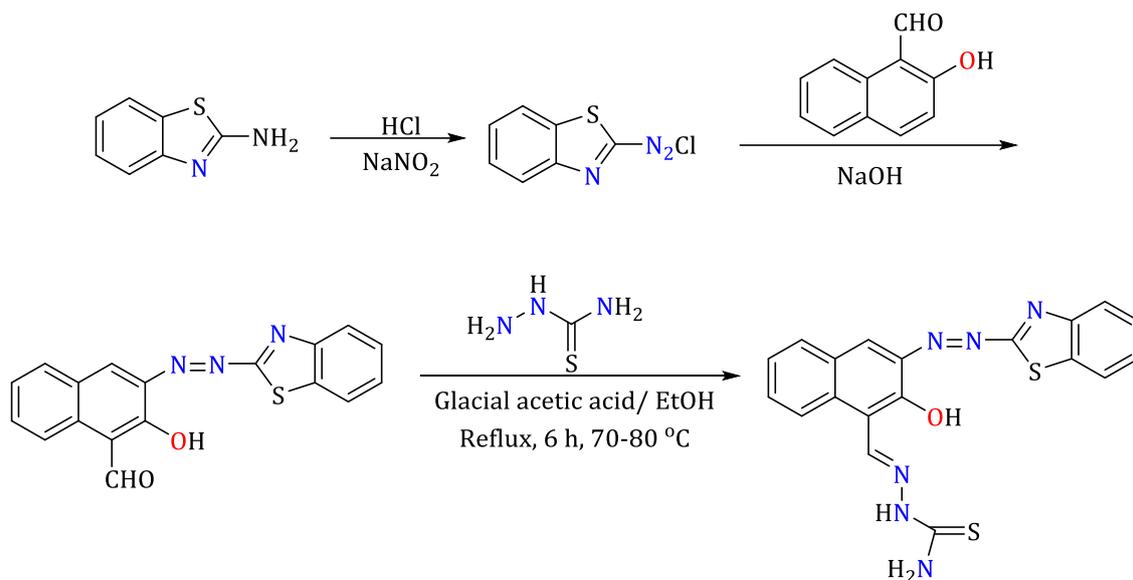
Materials and Methods

All material and chemicals obtained from Aldrich chemical company (USA) and Alfa Aesar and others. The ligands NMR spectra 1H -NMR and ^{13}C -NMR were evaluated in $DMSO-d_6$ with a Bruker 300 MHz instrument (300 MHz for 1H -NMR and 125 MHz for ^{13}C -NMR), using tetramethyl silane TMS as an internal reference. The FT-IR spectra as KBr discs were recorded using FT-IR-600 Fourier transform infrared spectra in the range $4000-400\text{ cm}^{-1}$. The electronic spectra of compounds were determined using a (UV-Vis) spectrophotometer type Shimadzu UV-160 in the range 1000-200 nm. The samples were solvated in DMSO using a

1.0 cm length cuvette cell with a concentration of $10^{-3}\text{ mol. L}^{-1}$ at room temperature. Mass spectra were obtained by (+) electrospray technique using Agilent mass spectrometer Sciex ESI MS. Melting points of prepared compounds were recorded using an electrothermal Stuart apparatus, model SMP 40 US. Magnetic moments at 308 K were measured with a magnetic susceptibility balance on Sherwood Scientific Devised. The complexes' molar conductivities were measured at room temperature using Eutech instruments cyberscan con (510) digital conductivity meter for (10^{-3} – 10^{-5} M solutions) of the compounds in Dimethyl sulfoxide solution (DMSO). Compounds were tested against four different bacteria and one species of fungi.

Synthesis of HL

Synthesis of thiosemicarbazide ligand [2-((4-(benzo[d]thiazol-2-yl)diazenyl)-3-hydroxynaphthalen-2-yl)methylene] hydrazine carbothioamide] from azo derivative [(4-(benzo[d]thiazol-2-yl)diazenyl)-3-hydroxy-2-naphthaldehyde]] which synthesized by reaction of 2-amino benzothiazole (1.5 g, 9.9 mmol) and sodium nitrite (0.689 g, 9.9 mmol) combined in 10 mL of water, chilled to $0-5\text{ }^\circ\text{C}$ and then mixed with hydrochloric acid with vigorous stirring to prepare azo compound according to the literature [7]. The cooled solution of 2-hydroxyl-1-naphthaldehyde (1.7 g, 9.9 mmol) and NaOH (0.399 g, 9.9 mmol) was then added to the diazonium salt solution, and the precipitate formed at $pH=4$ was filtered and rinsed many times with cold water before being dried at $pH\ 6-7$. The orange precipitate was filtered out, yielding (2.221 g, 66.71%) with $MP\ 79-81\text{ }^\circ\text{C}$, after thiosemicarbazide solution was added (0.274, 2.9 mm) to azo derivative (1 g, 2.9 mm) with a few drops of glacial acetic acid as a catalyst, refluxed 6 hrs, then left at room temperature extended to 15 minutes. The precipitate compound was obtained by filtration, washed with ethanol, and dried; light yellow product was collected. Yield: 0.894 g, 73.33 % $M.P>300\text{ }^\circ\text{C}$. The synthesis of the ligand [HL] is illustrated in [Scheme 1](#).

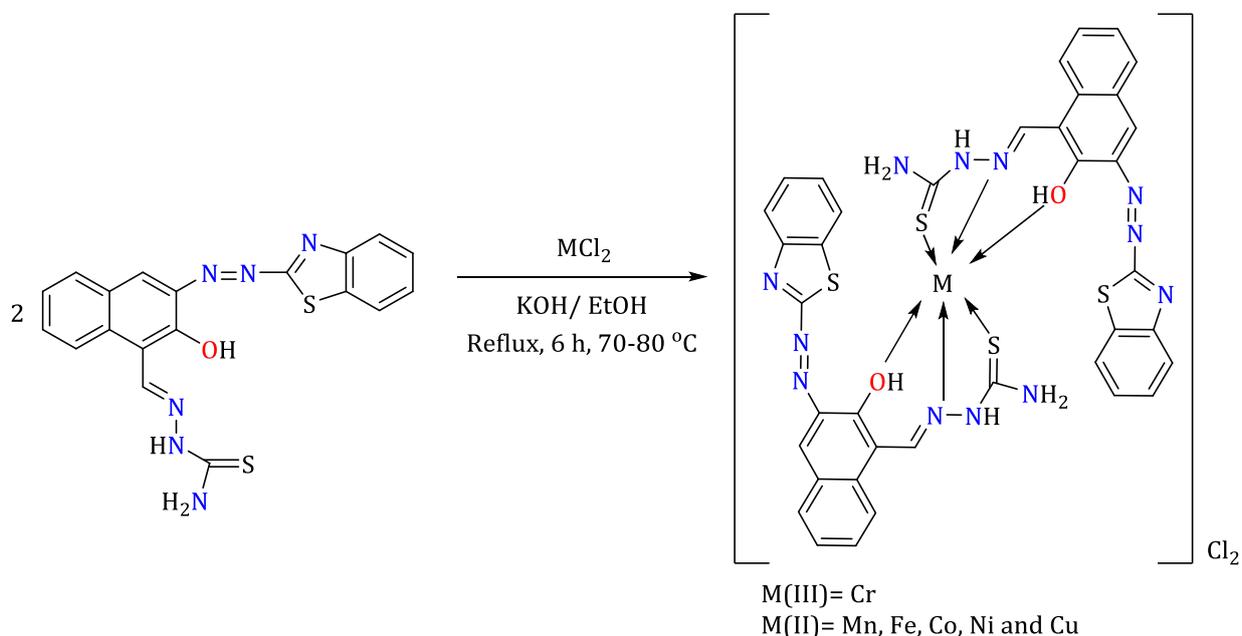


Scheme 1: Synthesis route for HL¹

Synthesis of complexes

The complexes were prepared by dissolving of HL ligand (0.4 g, 0.49 mmol) in 18 mL ethanol, followed by adding an equivalent amount from KOH (0.168 g, 0.49 mmol), followed by adding the metal salt CoCl_2 (0.0638 g, 0.49 mmol) as dropwise on the mixture of the ligand; the

mixture was refluxed for 6 hrs with continuous stirring at fixed temperature 70-80 °C. The mixture was filtered, and the resulted particulate was washed several times with cold ethanol; other complexes were prepared using the same method. [Scheme 2](#) shows the Synthesis route for metal complexes. Melting point of Complexes, colors and yields are all included in [Table 1](#).



Scheme 2: Synthesis route of metal complexes

Table 1: Metal salts quantities, colours, yields and melting points of complexes of HL¹

Complex of Metal ions	Weight of metal salt (g)	Complex weight (g)	Color	mp (°C)	Yield (%)
[Cr(HL) ₂]Cl ₃	0.14	971.32	Yellow	> 300	81
[Mn(HL) ₂]Cl ₂	0.10	938.81	light brown	> 300	75
[Co(HL) ₂]Cl ₂	0.07	942.80	Dark Brown	> 300	85
[Ni(HL) ₂]Cl ₂	0.12	942.56	Brown	> 300	77
[Cu(HL) ₂]Cl ₂	0.09	947.41	Green	> 300	81

Results and Discussion

All complexes were synthesized by reacting the metal salts with the Schiff base in a 1:2 mole ratio (M: L). All of the complexes were colored solid compounds. Table 1 shows that the analytical findings corresponded with the proposed

formula of a 1:2 M: L molar ratio. The complexes synthesized using HL were soluble in dimethyl form amide and dimethyl sulfoxide, which has partial solubility in ethanol, methanol, benzene, and water. Some of the physical and chemical characteristics are shown in Table 2.

Table 2: Elemental microanalysis data and some physical properties for complexes

Complex	M.Wt	Elemental analysis found %					
		M	C	H	N	S	Cl
[Cr(HL) ₂].Cl	971.32	5.35	46.99	2.91	17.30	13.2	10.95
		5.32	46.96	2.90	17.18	13.0	10.92
[Mn(HL) ₂]Cl ₂	938.81	5.85	48.62	3.01	17.90	13.66	7.55
		5.83	48.59	3.00	17.88	13.61	7.51
[Co(HL) ₂]Cl ₂	942.80	6.25	48.41	2.99	17.83	13.60	7.52
		6.22	48.39	2.95	17.80	13.57	7.50
[Ni(HL) ₂]Cl ₂	942.56	6.23	48.42	2.99	17.83	13.61	7.52
		6.19	48.40	2.96	17.79	13.59	7.49
[Cu(HL) ₂]Cl ₂	947.41	6.71	48.17	2.98	17.74	13.54	7.48
		6.68	48.12	2.93	17.70	13.50	7.42

FT-IR spectrum

Table 3 displays the spectrum of precursor (azo); the band at 3159 cm⁻¹ is referred to as the phenolic OH group. Bands at 3074 cm⁻¹ and 2889 cm⁻¹ referred to $\nu(\text{C-H})_{\text{aro}}$ and $\nu(\text{C-H})_{\text{ald}}$ groups respectively [8], the bands at 1643, 1627, (1608), 1465 and 1315 cm⁻¹ referred to C=N, C=O, C=C, N=N and C=S groups respectively. Also, in Table 3, numerical values of Schiff base ligand showed a band at 3448 cm⁻¹ and 3375 cm⁻¹ that refers to OH and NH groups, respectively, in HL and 3263 and 3167 cm⁻¹ referred to NH₂ attributed to asymmetrical and symmetrical stretching bending respectively. Also, bands appeared at 3051 and 2978-2908 cm⁻¹ that were assigned to $\nu(\text{C-H})_{\text{aro}}$ and $\nu(\text{C-H})_{\text{imc}}$, the bands at 1624, 1608, 1465, and 1361 may be assigned to C=N, C=C, N=N, and C=S groups respectively [8, 9]. The

spectrum of the complexes showed shifting in the imine band, phenolic band, and thione band, the shifting range was 1620-1612 cm⁻¹ in the imine [9], and the C-O shifting range was 3468-3429 cm⁻¹ [9, 10], while the thione band from 1361-1300. The azo group did not show any noticeable shift, which means the group did not participate in the coordination with the metal ions. Also, new bands for stretching frequencies of M-N have been allocated at 596-570 cm⁻¹, and another band was observed at range 497-470 cm⁻¹ indicating the formation of M-O coordination bond and range 455-420 cm⁻¹ ascribed M-S bond [8-10].

¹H and ¹³C -NMR spectra of the ligands

According to the literature review, the NMR spectroscopy was required to determine the structure of various types of compounds; NMR

spectra were recorded in DMSO-*d*₆ (dimethyl sulfoxide) using TMS (tetramethyl silane) as standard.

The ¹H-NMR spectrum of HL is shown in Table 4; the singlet signal of the phenolic proton OH appeared at 11.42 ppm. The small signal also appears as a singlet in 10.52 ppm may be assigned for NH, and the signal at 9.04 ppm may be attributed to the NH₂ group [8]. The spectrum of Schiff's base showed a peak at 8.55 ppm, which indicates the formation of the azomethine group (-CH=N) [11]. The multiple chemical shifts

around 7.19-8.55 ppm may be assigned to aromatic protons [12]. The ¹³C-NMR spectrum of HL, Table 5 in DMSO-*d*₆ solvent shows a chemical shift at the range 129.69-146.47 ppm can be attributed to C-S, C-O, C-N and C-C groups [9]. The chemical shift at 152.23 is attributed to azomethine endo, while 157.67 ppm can be attributed to azomethine group (C=N) [10]. The chemical shift in the range 109.67-128.99 could be attributed to the C aromatic group [9]. Finally, the chemical shift at 183.34 ppm can be attributed to the C=S group [8, 10, 11].

Table 3: The FT-IR spectral data of compounds (cm⁻¹)

Compound	V(OH)	NH	NH ₂ sy asy	νC-H arom	CH ald	C=N	νC=C νN=N	νC=S C-S	νC-O	νM-N	νM-O	νM-S
HL	3448	3375	3263 3167	3051	2978 2908	1624	1608 1465	1361 883	1238	-	-	-
[Cr(HL) ₂]Cl ₃	3469	3402	3283 3156	3068	2974 2816	1612	1588 1450	1314 821	1292	590	493	420
[Mn(HL) ₂]Cl ₂	3429	3367	3278 3178	3059	2916	1616	1535 1458	1342 825	1296	570	486	447
[Co(HL) ₂]Cl ₂	3468	3417	3263 3155	3062	2978 2927	1612	1597 1454	1311 821	1292	590	497	455
[Ni(HL) ₂]Cl ₂	3452	3402	3317 3155	3039	2904 2835	1620	1600 1450	1303 817	1248	570	470	420
[Cu(HL) ₂]Cl ₂	3437	3387	3332 3298	3032	2951 2839	1616	1600 1454	1300 813	1249	596	496	420

Table 4: ¹H-NMR spectral data for the ligand HL¹

Compound	Functional Group	δ (ppm)
HL ¹	O-H	(11.42) (1H, S)
	NH	(10.52) (1H, S)
	NH ₂	(9.04) (1H, S)
	CH _{ald}	(8.55) (1H, S)
	Ar-H	(7.19-8.53) (9H, m)

Table 5: ¹³C-NMR spectral data for the ligand HL¹

Compound	Functional Group	δ (ppm)
HL ¹	C=S	183.34
	C=N	152.23-157.67
	C-S, C-O, C- N and C-C	129.69-146.47
	C=C _{arom}	109.67-128.99

Mass spectroscopy

Table 6 shows the Schiff base ligand's mass spectrum, which has a well-defined molecular ion peak at m/z = 406.484 amu, which correlates (M⁺) with the Schiff base ligand's molecular

formula (C₁₉H₁₄N₆OS₂). The spectra of the HL ligand reveal a succession of peaks at m/z, 380.46, 264.33, 174.20, and 120.15, amu, which correspond to the fragments. The strength of these peaks indicates the pieces' stabilities [13].

Table 6: The mass fragmentation data of HL¹

No.	Fragment	Mass/Charge (m/z ⁺)
1	C ₁₉ H ₁₄ N ₆ O S ₂	406.48
2	C ₁₈ H ₁₄ N ₅ OS ₂ ⁺ [M ⁺¹]	380.46
3	C ₁₅ H ₁₀ N ₃ S ⁺	264.33
4	C ₈ H ₄ N ₃ S ⁺	174.20
5	C ₆ H ₂ NS ⁻	120.15

UV-Vis and the magnetic susceptibility measurement

In Table 7, the ligands' absorption spectra revealed peaks at 265, 329, and 381 nm due to the π→π* and 345 related to n→π* transition [2].

Table 7: Electronic spectral data of the ligand and its complexes

Complex	λ _{nm}	Wavenumber V(cm ⁻¹)	Molar extinction coefficient max (dm ³ mol ⁻¹ cm ⁻¹)	Assignment	Suggested geometry
HL ¹	265 329 345 381	37735 30395 28985 26246	1122 1510 2104 1398	π→π* π→π* n→π* π→π*	-
[Cr(HL) ₂]Cl ₃	268 301 431 679 848	37313 33222 23201 14727 11792	816 699 114 7 3	Intra ligand Intra ligand C.T ⁴ A _{2g(F)} → ⁴ T _{2g(F)} ⁴ A _{2g(F)} → ⁴ T _{2g(F)}	Octahedral
[Mn(HL) ₂]Cl ₂	275 345 416 734 780 821	36363 28985 24038 13623 12820 12180	2142 2070 1506 45 42 39	Intra ligand Intra ligand C.T ⁶ A _{1g} → ⁴ T _{1g} ⁶ A _{1g} → ⁴ T _{1g} ⁶ A _{1g(S)} → ⁴ T _{1g(G)}	Octahedral
[Co(HL) ₂]Cl ₂	278 345 348 438 460 461 803	35971 28985 28735 22831 21739 21691 12453	2278 1715 2500 845 1581 806 7	Intra ligand Intra ligand Intra ligand C.T C.T ⁴ T _{1g(F)} → ⁴ T _{1g(P)} ⁴ T _{1g(F)} → ⁴ T _{2g(F)}	Octahedral
[Ni(HL) ₂]Cl ₂	271 273 314 320 385 389 431 432 760 855	36900 36630 31847 31250 25974 25706 23201 23148 13157 11695	1849 2059 2031 1761 1331 1469 660 1089 22 22	Intra ligand Intra ligand Intra ligand Intra ligand Intra ligand Intra ligand C.T C.T ³ A _{2g(F)} → ³ T _{1g(F)} ³ A _{2g(F)} → ³ T _{1g(P)}	Octahedral
[Cu(HL) ₂]Cl ₂	271 325 387 429 760 799	36900 30769 25839 23310 13157 33444	1862 1679 1051 1115 31 26	Intra ligand Intra ligand Intra ligand ² B _{1g} → ² E _g ² B _{1g} → ² A _{1g} ² B _{1g} → ² A _{1g}	D. Octahedral

The range of peaks at 271-387 nm may be related to intra ligand for complexes **1-5** [14], while the range of peaks at 416-438 nm revealed to (CT) for complexes [8-10]. Finally, the peaks in the visible region can be associated with d-d transitions. The electronic spectrum of Co(II) displays the peaks at 461 and 803 nm related to ${}^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}$ and ${}^4T_{1g(F)} \rightarrow {}^4T_{1g(F)}$ transitions, respectively, octahedral geometry around the central metal ion was suggested [14]. The electronic spectrum of Cu(II) complex displays peaks at 429 nm may be revealed to ${}^2B_{1g} \rightarrow {}^2E_g$ transition, and at 760-799 related to ${}^2B_{1g} \rightarrow {}^2A_{2g}$ transition, which revealed that the complex own octahedral geometry [14, 15]. The electronic spectrum of Mn(II) displays the peaks at 734, 780, and 821 nm related to ${}^6A_{1g} \rightarrow {}^4T_{1g}$ and ${}^6A_{1g(s)} \rightarrow {}^4T_{1g(G)}$ transition, the octahedral geometry around the complex was suggested [16, 17]. The electronic spectrum of Cr(III) displays the peaks at 679 and 848 nm related to ${}^4A_{2g(F)} \rightarrow {}^4T_{2g(F)}$ [7, 8], and octahedral geometry was proposed [9]. The electronic spectrum of the

Ni(II) complex displays the peak at 855 nm related to ${}^3A_{2g(F)} \rightarrow {}^3T_{1g(P)}$, which suggests an octahedral geometry around the central metal ion [14]. The magnetic moment of the complexes Ni(II), Cu(II), Co(II), Mn(II), and Cr(III) value agrees with the electronic spectrum transition and the suggested structures.

Biological activities

The effect of the synthesized compounds was evaluated using the agar diffusion technique on four types of bacteria (*E. Coli*, *Enterobacter S aureus*, and *B. Subtilis*) and one type of fungi (*Candida*) (Table 8). The inhibition zone (IZ) of 1×10^{-3} M of the compounds was compared to the IZ of Ceftriaxone (BP) antibiotic as standard [18, 19]. The solvent was conducted, and its effect on bacterial growth -ve (zero) [20]. The bacteria and fungi were chosen for their recognized impact on various illnesses; they exhibit varying resistance to medicines and medicinal compounds. The ligand and its complexes demonstrated a reasonable zone of inhibition.

Table 8: The assessment of ligands and their complexes from biological perspective

Complex	Klebseilla pneumoniae (G-)	Pseudomonas aeruginosa (G-)	Bacillus subtilus (G+)	Staphylococcus aureus (G+)	Candida Albicans
HL	13	16	8	16	16
[Cr(HL) ₂]Cl ₃	10	17	8	12	17
[Mn(HL) ₂]Cl ₂	15	18	8	12	17
[Co(HL) ₂]Cl ₂	13	10	8	10	20
[Ni(HL) ₂]Cl ₂	10	10	10	22	20
[Cu(HL) ₂]Cl ₂	14	15	8	12	20

Conclusion

It is reported that the synthesis and characterization of azo Schiff base ligand by reacting (4-(benzo [d] thiazol-2-ylidiazonyl)-3-hydroxy-2-naphthaldehyde) with thiosemicarbazides to produce HL. The ligand interaction with metal salts to produce complexes more stable the imine group atoms (N), (OH), and (C=S) performed as tridentate coordinates. Uses such as FT-IR, UV-Visible, ¹³C, ¹H-NMR, molar conductivity, and mass, magnetic, susceptibility indicated that all complexes were produced to adopt an octahedral geometric form.

When tested for biological effectiveness against four types of bacteria (*E. Coli*, *Enterobacter S aureus*, and *B. Subtilis*) and one type of fungi (*Candida*), all of the synthesized compounds showed adequate inhibition in various ranges against the bacterium spices; also, they show the effect on the fungi spices.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

There are no conflicts of interest in this study.

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