SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL, AND ANTICANCER ACTIVITY OF 2-{[4-(3,5-DIMERCAPTO-[1,2,4]TRIAZOLE-4-YLAZO)-PHENYL]-HYDRAZONOMETHYL}-PHENOL AND ITS METAL COMPLEXES

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ABSTRACT

Transition metal complexes of Co (II), Ni (II), Cu (II) and Zn (II) with a newly designed ligand 2-{[4-(3,5-dimercapto-[1,2,4]triazole-4-ylazo)-phenyl]-hydrazonomethyl}-phenol has been synthesized and analyzed by numerous spectro-analytical techniques like elemental analysis, infrared, nuclear magnetic resonance, mass, ultraviolet-visible spectral analysis. The newly synthesized ligand and metal complexes have been evaluated for their growth-inhibiting ability against anticancer activity, fungal and bacterial strains. The results have shown that both the ligand and their metal complexes inhibit the organism's growth. However, the metal complexes had shown more activity than the ligand in all the activities. This shows that the compounds anticancer, antifungal and antibacterial activity increases by the chelation or coordination.

Keywords: Azo Compounds, Chelation, Diazotization, Phenyl Hydrazine, Spectro-analytical Techniques, In-vitro Anticancer Activity.

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INTRODUCTION

Azo compounds are synthesized with an intermediate diazonium salt. They have huge applications in pharmaceutical,¹ food,² textile,³ printing,⁴ and the cosmetic industries.⁵ The development of azo compound based on heterocyclic compounds is an important task for heterocyclic chemists, nitrogen and sulfur-containing five-membered heterocyclic compounds show numerous therapeutic areas.⁶⁻⁷ Azo compounds are the largest and most versatile class of compounds and are characterized by the presence of the chromophoric azo group (-N=N-).⁸ These are synthesized by diazotization and coupling reactions, firstly diazotization of the primary amine will give the diazonium intermediate salt, and this intermediate compound gives azo compound by coupling. Coordination compounds have developed in diverse disciplines because these compounds are used in various biological applications.⁹ Azo compounds have often been used as chelating compounds in coordination chemistry, and their metal chelates have been of great interest. The present work includes the synthesis of diazo compounds using 3,5-dimercapto-4-amino-1,2,4-triazole, and its different metal complexes using Schiff base ligand.

EXPERIMENTAL

Material and Methods

All the chemicals were obtained from Merck, Sigma-Aldrich, SD fine and used without any further purification. Completion of reaction is tested by TLC (Thin-layer chromatography).¹⁰ All the synthesized compound's characterizations were recorded by using elemental analysis, IR, UV, NMR and mass spectra. IR spectrum of the ligands and metal complexes were obtained by the KBr pellet method on a Perkin Elmer-FTIR spectrometer; An Electronic spectrum of the ligand and metal complexes was recorded using the Ocean Optics UV-visible Spectrometer. The ¹H NMR spectrum was recorded using

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CDCl_3 as a solvent on a Bruker 400 MHz spectrometer with TMS (Tetramethyl silane) as an internal standard (Chemical shift in δ ppm). The mass spectrum was recorded on LCMS on Shimadzu mass spectrometer.

Synthesis of 3,5-dimercapto-4-amino-1,2,4-triazole

A mixture of thiocarbohydrazide (0.1 mol) and carbon disulfide (0.2 mol) was refluxed in pyridine (80 mL) for 1 h, yellow-colored solid separated was filtered and dissolved in 20 mL of hot water followed by the drop by drop addition of concentrated HCl (3 mL). The solid was separated and recrystallized using distilled water.¹¹

Synthesis of azodyes (4-(4-hydrazine-phenyl-diazenyl)-4H-[1,2,4] triazole-3,5-dithiol

The 3,5-dimercapto-4-amino-1,2,4- triazole (2 mmol) was dissolved in concentrated HCl (5-6 mL) cooled in a freezing mixture and to this, a cold solution of NaNO_2 (2 mmol) was drop-wise added with stirring. The resulting mixture was stirred for 3 h at 0-5 $^\circ\text{C}$. The cold solution of diazonium salt intermediate was added to the coupling agent phenylhydrazine (2 mmol), and the reaction mixture was stirred for another 2 h at 0-5 $^\circ\text{C}$. The solid separated was filtered off, washed with distilled water, dried and recrystallized using ethanol.

Synthesis of Schiff base ligand (2-{{4-(3, 5-dimercapto-[1,2,4] triazole-4-ylazo)-phenyl}-hydrazonomethyl}-phenol)

The azo compound (1 mmol) dissolved in ethanol and salicylaldehyde (1 mmol) was added with a drop of acetic acid as a catalyst. Reflux the reaction mixture for 4 h and the completion of the reaction is tested by TLC. After completing the reaction, the solution was poured in ice-cold water, filtered the precipitate, and recrystallized with alcohol.

Synthesis of Complex

The ligand 2-{{4-(3,5-Dimercapto-[1,2,4]triazole-4-ylazo)-phenyl}-hydrazonomethyl}-phenol (2 mmol) was dissolved in 20 mL methanol in a dry round bottom flask. The metal salts (Co (II), Ni (II), Cu (II) and Zn (II) acetates) were dissolved in a minimum amount of methanol. To this methanolic solution of metal salt (1 mmol), the hot solution of ligand was added drop-wise and refluxed for 3 h. The solid separated was filtered off, washed with cold methanol and dried.

RESULTS AND DISCUSSION

Scheme of the Reaction

The schematic representation of the synthesis of the ligand is shown in Fig.-1.

Elemental Analysis

All the compounds were analyzed for carbon, hydrogen, nitrogen and sulfur on a CHNS analyzer. The estimation of metal in the complex was carried out by a standard method.¹² The elemental analysis of the synthesized ligand and its complexes is as follows in Table-1.

Table-1: Elemental Analysis of Ligand and Its Metal Complexes

Ligand/Complex	C (%) (Calculated)	H (%) (Calculated)	N (%) (Calculated)	S (%) (Calculated)	M (%) (Calculated)
$\text{C}_{15}\text{H}_{13}\text{N}_7\text{OS}_2$	49.70 (48.51)	3.69 (3.52)	26.93 (26.40)	16.14 (17.24)	-
$(\text{C}_{15}\text{H}_{13}\text{N}_7\text{OS}_2)_2\text{Co}$	46.30 (44.95)	3.41 (3.26)	23.38 (24.46)	16.03 (15.97)	8.06 (7.35)
$(\text{C}_{15}\text{H}_{13}\text{N}_7\text{OS}_2)_2\text{Ni}$	45.79 (44.96)	4.01 (3.36)	26.48 (24.47)	15.58 (15.97)	7.94 (7.32)
$(\text{C}_{15}\text{H}_{13}\text{N}_7\text{OS}_2)_2\text{Cu}$	45.26 (44.69)	3.44 (3.24)	25.56 (24.32)	14.82 (15.88)	8.21 (7.88)
$(\text{C}_{15}\text{H}_{13}\text{N}_7\text{OS}_2)_2\text{Zn}$	46.74 (44.59)	3.36 (3.24)	25.28 (24.26)	16.42 (15.84)	8.67 (8.09)

Infrared Spectra

In the FTIR spectra, the free ligand shows a relatively sharp band at 3417 cm^{-1} . This is assigned to ν (O-H) of the ligand. This band has significantly broadened in all the metal complexes, indicating the coordination of oxygen atoms without deprotonation.¹³ The absorption band at 1617 cm^{-1} is due to the ν (C=N) (azomethine) group, the primary ν (N-H) stretching vibrations have been observed at 3321 cm^{-1} .

CC(C)(C)C(F)(F)F.N#Cc1cc(Cl)cc(Cl)c1.Cl[N+](=O)[O-].[Na+].Cl

 + NaNO₂ + HCl +
 ClC1=CC(=CC=C1C#N)Cl

 ↓ Stirring 5 h
0-5 °C

CC(C)(C)C(F)(F)F.N#Cc1cc(Cl)cc(Cl)c1 + ClC1=CC(=CC=C1C#N)Cl

 ↓ Reflux 4 h

CC(C)(C)C(F)(F)F.N#Cc1cc(Cl)cc(Cl)c1

10

Table-2: Stretching Frequencies of IR Spectrum in cm^{-1}

Ligand/Complex	O-H	N-H	C-H	S-H	C=N	N=N	M-O	M-N
C ₁₅ H ₁₃ N ₇ OS ₂	3417	3321	3058	2834	1617	1495	—	—
(C ₁₅ H ₁₃ N ₇ OS ₂) ₂ Co	3426	3319	3062	2845	1602	1484	519	442
(C ₁₅ H ₁₃ N ₇ OS ₂) ₂ Ni	3426	3321	3049	2837	1601	1482	514	420
(C ₁₅ H ₁₃ N ₇ OS ₂) ₂ Cu	3435	3320	3054	2840	1593	1477	511	437
(C ₁₅ H ₁₃ N ₇ OS ₂) ₂ Zn	3443	3317	3056	2831	1594	1486	503	429

The exhibited signals of all protons of the Schiff base ligand are found to be in their expected regions (Fig.-2).¹⁹ The ¹H NMR spectrum of the ligand shows the peaks at δ 2.50 ppm (singlet) is due to S-H, δ 3.304 ppm (singlet) is due to the amine protons, and the aromatic protons in the region of δ 6.67-7.76 ppm,²⁰ azomethine proton (HC=N) at δ 8.08 ppm (singlet), phenolic (Ar-OH) peak in the region of δ 10.49 ppm (singlet), and finally hydrazone protons at δ 11.39 ppm (singlet).

The mass spectrum of the ligand shows (Fig.-3) the molecular weight of the ligand 371.45 m/z and the calculated molecular weight was 371.39. In this mass spectrum, the M^+ peak value is 371.45 and the

fragmentations take place at 278.88, 244.93, 187.86 and 151.89 due to loss of phenyl, thionyl, triazole and hydrazinyl groups, respectively.

Fig.-2:¹H NMR Spectrum of the Ligand

Fig.-3: Mass Spectrum of the Ligand

Electronic Spectra

The Schiff base ligand shows two absorption bands in the region 262 and 363 nm was due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively, in Table-3. In the copper metal complex, the absorption bands in the region 283, 405 and 543 nm were due to the $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and ${}^2E_g \rightarrow {}^2T_{2g}$ transitions, respectively. The forbidden ligand $\rightarrow$ metal charge transfer is due to John -Teller distortion, suggesting distorted octahedral geometry around the Cu (II) ion.²¹ In the cobalt metal complex the observed absorption bands at 292, 376 and 601 nm were assigned to $\pi \rightarrow \pi^*$, ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ and ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}$ transitions, respectively and octahedral geometry suggesting for the Co(II) ion.²² In the nickel complex the absorption bands at 278, 337 and 522 nm the transitions are assigned to $\pi \rightarrow \pi^*$, ${}^3A_{2g} \rightarrow {}^3T_{2g}$ and ${}^3A_{2g} \rightarrow {}^3T_{1g}(P)$, respectively, which shows octahedral geometry for the Ni (II) complex.²³⁻²⁴ Finally, the zinc complex only shows the $\pi \rightarrow \pi^*$ transition at 262 nm and $n \rightarrow \pi^*$ transition at 350 nm.

Magnetic Susceptibility

The magnetic moment of the Cu (II), Co (II) and Ni (II) complexes were measured using Gouy balance at room temperature.²⁵ The magnetic moment of the Cu (II) complex was observed 1.78 BM corresponding to one unpaired electron, due to John-Teller distortion the geometry of the complex was distorted octahedral geometry.²⁶ The magnetic moment of Co (II) was 3.82 BM and finally, the Ni (II) complex was 2.95 BM and the metal Zn is diamagnetic.²⁷⁻²⁸

Table-3: Electronic Spectral Data of the Schiff base Ligand and Metal Complexes

Ligand/ Complex	Absorption Bands (nm)	Assigned Transitions
C ₁₆ H ₁₆ N ₈ O ₂	262, 363	$\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$
Co(C ₁₆ H ₁₆ N ₈ O ₂) ₂	292, 376, 601	$\pi \rightarrow \pi^*$; ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$; ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}$
Cu(C ₁₆ H ₁₆ N ₈ O ₂) ₂	283, 405, 543	$\pi \rightarrow \pi^*$; $n \rightarrow \pi^*$; ${}^2E_g \rightarrow {}^2T_{2g}$
Zn(C ₁₆ H ₁₆ N ₈ O ₂) ₂	262, 350	$\pi \rightarrow \pi^*$; $n \rightarrow \pi^*$
Ni(C ₁₆ H ₁₆ N ₈ O ₂) ₂	278, 337, 522	$\pi \rightarrow \pi^*$; ${}^3A_{2g} \rightarrow {}^3T_{2g}$; ${}^3A_{2g} \rightarrow {}^3T_{1g}(P)$

Antibacterial Activity

A metal chelates exhibit higher activity than ligands.²⁹ This was based on the inhibition of the growth of bacteria by a measured concentration of the antibiotics to be examined with a known concentration of the standard drug.³⁰ The synthesized compounds were screened for their antibacterial activity by using the agar disc diffusion method. The common bacteria *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used in this method. The ligand and its complexes were taken in the concentrations 5, 10, 25, 50 and 75 µg/mL. The agar media were prepared in distilled water. The known concentrations of antibiotics are placed on agar plates and inoculated uniformly at 37 °C for 18-24 h. In these prepared agar plates, the wells were made for sample filling. In different concentrations, the wells were filled with samples. All the plates were incubated at 37 °C for 48 h and the inhibition zone was noted. The ligand results were moderately active against the bacteria, and the complexes were more active when compared to a ligand. Between the Gram-positive and Gram-negative bacterial strains, the synthesized compounds have shown higher inhibition growth against *S. aureus* (Gram-positive bacteria strain). The results are as follows in Fig.-4.

Fig.-4: Antibacterial Activity of the Schiff base Ligand and Its Metal Complexes

Antifungal Activity

Antifungal activity of the synthesized compounds was done by agar diffusion methods using *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 10549). The concentrations of the compounds were 5, 10, 25, 50 and 75 µg/mL. The stock cultures of fungi were prepared by inoculating in broth media and grown at 37 °C for 18-24 h. In these prepared agar plates, the wells were made for sample filling, wells were filled with the sample in different concentrations.³¹ At 37 °C the plates were incubated for 48 h. The zone of inhibition was measured. The results were shown very good inhibition growth against the fungal strains, as shown in Fig.-5.

Anticancer Activity

In vitro cytotoxicity of synthesized Schiff base ligand and its metal complexes was applied against the MCF-7 (human breast cancer cell lines) by MTT assay using cisplatin as a standard. The cytotoxic effects are induced by the synthesized compounds in a concentration-dependent manner, as shown in Fig.-6, Table-5.

Fig.-5: Antifungal Activity of the Schiff base Ligand and Its Metal Complexes

The cells were seeded a 96-well flat-bottom microplate and maintained at 37 °C in 95% humidity and 5% CO₂ overnight. Different concentrations (400, 200, 100, 50, 25 and 12.5, µg/mL) of samples were treated and incubated for 48 h.³² “The wells were washed twice with PBS and 20 µL of the MTT staining solution was added to each well and the plate was incubated at 37 °C. After 4 h, 100 µL of DMSO was added to each well to dissolve the formazan crystals and absorbance was recorded with 570 nm using a microplate reader”. The results of the compounds have shown that all the compounds exhibited significant activity against the MCF-7 cell line. The IC₅₀ values of the ligand and metal complexes exhibit a very strong activity towards cancer cell line MCF-7 with the values of 7.768, 8.614 and 4.356 µg/mL, respectively (Table-4). In this study, the Cu (II) complexes show a very good activity than the other. There are plenty of reports available in the literature that pinpoints the enhanced anticancer activity of Cu (II) complexes.

Table-4: Cytotoxic Activity of the Ligand and Metal Complexes against Cancer Cells

Compound	IC ₅₀ Values (µM) MCF-7
Ligand	7.768
Co (II)	8.614
Cu (II)	4.356
Cisplatin	2.200

IC₅₀ (µM): 1-10 (very strong), 11-20 (strong), 21-50 (moderate), 51-100 (weak) and above 100 (non-cytotoxic).

Fig.-6: Cytotoxic Studies of the synthesized Compounds with MCF-7 Cancer Cell Line by MTT Assay

Table-5: Percentage Cell Viability at Different Concentrations of the Compounds.

Concentration (µg/mL)	A (ligand)		B (Cu complex)		C (Co complex)	
400	23.61	22.63	24.82	15.33	23.85	25.07
200	24.82	25.55	27.5	25.31	26.53	27.01
100	26.04	27.26	31.88	31.15	29.2	30.42
50	27.26	28.47	37.24	35.78	33.83	32.37
25	34.56	38.21	42.35	40.16	37.72	40.16
12.5	50.13	52.81	47.21	44.78	41.86	46.00

M = Co (II), Cu (II), Ni (II) and Zn (II) metal ions

Fig.-7: Proposed Structure for the Metal Complex

CONCLUSION

The metal complexes show higher antimicrobial activity and anticancer activity than the ligand because of the chelation. Based on the elemental analysis of the metal complexes, the ratio of metal to the ligand is 1:2 in all the complexes. The ligand environment forms an octahedral geometry around the metal centers and as shown in the proposed structure (Fig.-7). The ligand and its metal complexes show very good activity against bacterial and fungal strains. The anticancer activity of the analyzed compounds shows very good activity, whereas; copper complex shows strong activity than the remaining compounds.

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