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NEW METAL COMPLEXES OF AZO LIGAND BASED ON A MONOTERPENE CORE: SYNTHESIS, STRUCTURAL CHARACTERIZATION, ANTIOXIDANTS, AND DNA-CLEAVAGE ACTIVITY

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ABSTRACT. New mononuclear complexes of Cr(III), Fe(III), Zn(II), Mo(IV), and Cd(II) have been designed and synthesized, utilizing a tridentate azo ligand, 1-(5-allyl-2-hydroxy-3-methoxyphenyl)diazenyl)anthraquinone (H-L), derived from amino anthraquinone and the phenolic monoterpene (eugenol), known for its diverse pharmacological characteristics. The free ligand and its metal chelates were characterized by various complementary spectroscopic, spectrometric, thermal, magnetic, and analytical techniques. According to the molar conductivity data, all complexes except the Mo(IV) complex behave as neutral species. The in vitro antioxidant activity, as estimated by the DPPH assay, showed concentration-dependent radical-scavenging activity for the ligand and its complexes, with the Cr(III) complex demonstrating potency comparable to that of the standard (Trolox). DNA-cleavage studies revealed pronounced activity for the Cr(III), Fe(III), Zn(II), and Mo(IV) complexes in the absence of external redox agents. In contrast, the Cd(II) complex showed no apparent activity. These results reveal that anthraquinone-natural monoterpene (eugenol)-based azo ligand serves as an effective scaffold for bioactive coordination compounds that function as DNA-targeting and antioxidant agents, thereby suggesting their potential as promising candidates for future biological applications.

KEY WORDS: Azo ligand, Antioxidant, DNA cleavage, Metal complexes, Monoterpene (Eugenol)

INTRODUCTION

Metals are crucial in biological systems and pharmaceutical chemistry due to their remarkable properties, including charge, tendency to form coordinate bonds, Lewis acidity, partially filled d-orbitals, and redox activity [1]. Transition metals, in particular, are essential for numerous physiological processes, and several transition-metal-based drugs are currently in clinical settings [2]. Complexes with multidentate ligands are especially noteworthy because they can exist in multiple oxidation states, making them significant in biological inorganic chemistry and in redox systems involving enzymes [3]. Anthraquinone derivatives are significant natural compounds with diverse pharmacological activities, including antitubercular, antibacterial, and anti-HIV effects, as well as applications in the textile industry. In bioinorganic chemistry, their metal complexes are of great interest for possible medicinal development [4, 5]. Eugenol, a naturally occurring phenolic monoterpene, exhibits cardiovascular, antidepressant, anticancer, and antioxidant properties, rendering it valuable for flavoring, medicinal, and fragrance applications [6]. Interestingly, azo dyes derived from eugenol exhibit antibacterial properties and show promising anticancer potential [7]. Azo dyes are known for their stability and vibrant colors, making them popular across various industries and attracting significant attention for their diverse applications [8, 9]. The coordination of azo dyes with metal ions is influenced by several factors, including the metal type, its oxidation state, the donating atoms, the formation of chelate rings, and the pH of the reaction medium. Azo dye-metal complexes exhibit a wide range of biological activities, including inhibition of protein, DNA, and RNA synthesis, and potential promotion of

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carcinogenesis [10, 11]. Additionally, many of these complexes possess antioxidant functions, effectively scavenging free radicals and thereby decreasing oxidative stress [12]. A significant aspect of this research is the interaction of these compounds with DNA; hydrolytic DNA cleavage, which breaks the phosphodiester bond without the need for external oxidants or reductants [13], offers a cleaner, controlled method than oxidative pathways. Natural nucleases have diverse active sites that contain various metal ions, making it challenging to elucidate their mechanisms [14]. As a result, synthetic metallic nucleases, such as azo dye-metal complexes, serve as beneficial models for mimicking nuclease activity and studying the cleavage of phosphodiester bonds [15]. Importantly, this cleavage activity has been linked to enhanced anticancer potential [13]. Overall, these characteristics make azo dye-metal complexes promising candidates for bioinorganic research, antioxidant therapy, and the development of anticancer drugs.

Azo-based metal complexes have been widely studied due to their various coordination chemistry and potential biological significance. However, metal complexes derived from azo ligands containing a monoterpene moiety are relatively underexplored. In particular, integrating a monoterpene core into an aminoanthraquinone-derived azo framework and subsequently investigating its corresponding metal complexes has not been reported extensively. Such hybrid ligand systems provide an interesting platform for exploring the relationships between azo, phenolic, and carbonyl functionalities and their possible roles in metal-binding behavior and biological activities. Therefore, this study focuses on the synthesis and characterization of an eugenol-containing azo ligand and its complexes with Cr(III), Fe(III), Zn(II), Mo(IV), and Cd(II). The ligand was synthesized by combining an aminoanthraquinone-derived azo framework with an eugenol moiety, providing a multifunctional coordination system. The resulting compounds were characterized using a combination of physical, analytical, and spectroscopic techniques to investigate their composition and proposed structure. Furthermore, the in vitro biological activities of both the free ligand (H-L) and its metal chelates were evaluated by assessing their antioxidant capacity and DNA-cleavage activity to provide insights into their potential biological relevance.

EXPERIMENTAL

Materials and methods

All chemicals utilized in this study were sourced from BDH, Merck, and Fluka. Elemental microanalyses (C, H, N) were conducted via a Eurovector version EA-3000, single-V-3.0. For the ligands, NMR spectra were recorded with a Bruker 400 MHz spectrometer in DMSO-d_6, using the internal standard (tetramethylsilane). An electrothermal apparatus was employed to assign the melting points of the azo ligand and its metal chelates. The metal content was determined using a Shimadzu AA680 flame atomic absorption spectrophotometer. A W-T-W conductivity meter was used to measure the molar conductance of the prepared complexes (10^{-3} M) in DMSO at 25 °C. Magnetic susceptibility measurements were obtained with the MSB-MK-1 model. Mass spectra for all compounds were acquired through a Shimadzu QP50A-DI Analysis QP-2010. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of the solid compounds were performed in a nitrogen atmosphere at a heating rate of 10 °C min^{-1}, over the temperature range of 25 to 600 °C using a Shimadzu TGA analyzer. UV-Vis spectra were obtained using a Shimadzu-1800 spectrophotometer with a quartz cell (200-1100 nm). IR spectra were measured using a spectrophotometer (Shimadzu FTIR-8400S), with KBr and CsI discs.

Synthesis of 1-(5-allyl-2-hydroxy-3-methoxyphenyl)diazenyl)anthraquinone (H-L) ligand and metal complexes

The preparation methods for the ligand and its metal chelates are detailed in the experimental section of the Supporting Information. Scheme 1 illustrates their synthetic pathways. For free ligand, ^{1}H-NMR (400 MHz, DMSO-d_6), (δ, ppm): 2.69 (Ar-OCH_3), 6.34-6.38 (CH=CH_2), 6.49-6.55 (m, Ar-H), 6.62 (d, Ar-H), 6.82 (s, Ar-H), 7.58 (d, Ar-H), 8.58 (d, Ar-H), 9.16 (Ar-OH); ^{13}C-NMR (100 MHz, DMSO-d_6), (δ, ppm): 22.36, 44,78, 75.78, 78.37, 98.54, 98.67, 98.89, 108.34, 108.54, 108.85, 118.11, 118.26, 119.06, 119.16, 125.31, 125.69, 134.86, 142.26, 144.75, 160.04, 180.30, 186.85, 195.31.

Biological methods (in vitro)

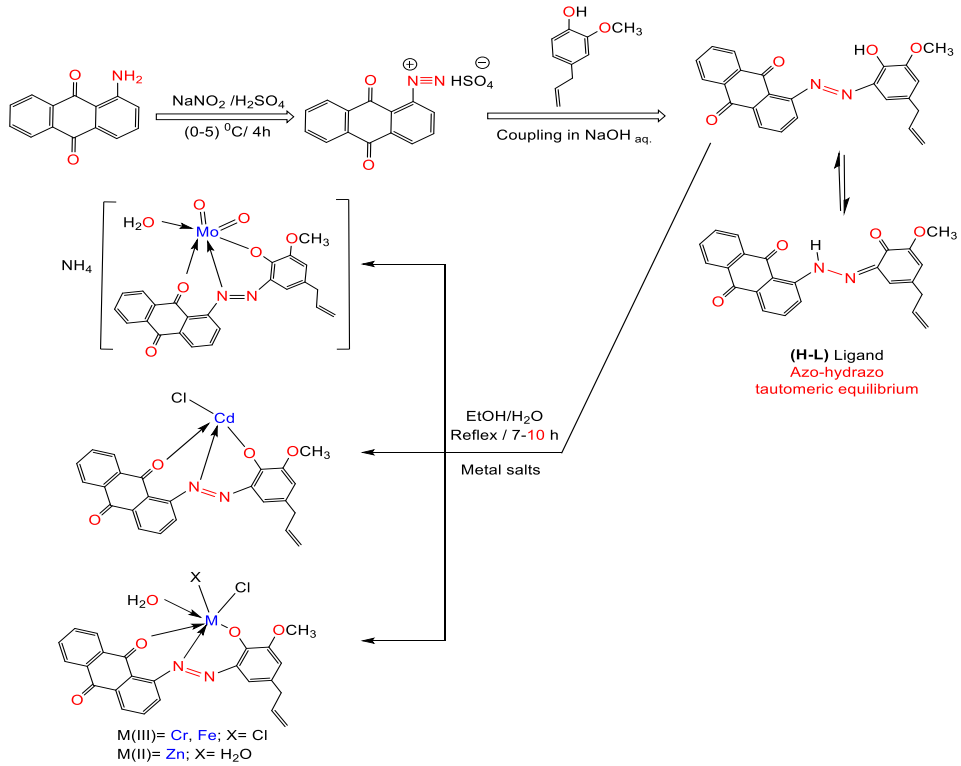
All biological experiments were carried out in compliance with standard laboratory biosafety and ethical guidelines. (See Supporting Information for the detailed experimental method).

Antioxidant (DPPH) studies

The antioxidant capacity of the free ligand and its corresponding metal chelates was evaluated in vitro using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay [16], with compound concentrations ranging from 12.5 to 200 µg/mL, and gallic acid and Trolox serving as reference standards. The samples were incubated with the DPPH solution at 37 °C for 30 minutes, and the absorbance was measured at 517 nm. IC_{50} values were calculated using GraphPad Prism software.

DNA-cleavage experiment studies

In accordance with established techniques [17], the culture media were prepared, and DNA was isolated for cleavage experiments. Using DNA from Proteus mirabilis (a Gram-negative bacterium) and Staphylococcus aureus (a Gram-positive bacterium) as targets, the potential of the synthesized ligand and its metal complexes to cleave DNA was evaluated by agarose gel electrophoresis. DNA samples were incubated with the test compounds (1.00 mg mL^{-1} in freshly distilled DMSO) at 37 °C for two hours. After incubation, electrophoresis was performed at 50 V for 30-45 min. The gels were stained using ethidium bromide (10 µg mL^{-1}), and DNA bands were visualized under UV illumination.



Scheme 1. Synthesis diagram of (H-L) ligand, suggesting tautomeric forms with proposed structures of related metal complexes.

RESULTS AND DISCUSSION

The azo H-L ligand and its metal chelates of Cr(III), Fe(III), Zn(II), Mo(IV), and Cd(II) were successfully synthesized and examined using various physicochemical techniques. Elemental analysis supported the suggested molecular formulations and a 1:1 metal-to-ligand (M:L) stoichiometry, revealing good agreement between the calculated and experimental values. Chloride ions were quantified using argentometric titration, while the metal content of the complexes was examined through atomic absorption spectroscopy. Table 1 summarizes the fundamental analytical and physicochemical data.

Table 1. Physical-analytical characteristics of the ligand (H-L) and its metal complexes.

Comp. Molecular formula (M.wt)	m.p.(°C) (Color)	Yield %	Elemental analysis found (Calculated)					Conductivity $cm^2\ \Omega^{-1}\ mol^{-1}$
			C %	H %	N %	M %	Cl %	
$C_{24}H_{18}N_2O_4$ (398.42)	186 - 188 (Dark plum)	75	71.88 (72.28)	4.45 (4.51)	8.17 (7.02)	–	–	–
$C_{24}H_{19}Cl_2N_2O_5Cr$ (538.32)	> 300 (Dark brown)	66	54.11 (53.54)	3.04 (3.56)	6.17 (5.20)	9.04 (9.66)	13.78 (13.17)	13

$C_{24}H_{19}Cl_2N_2O_5Fe$ (542.17)	> 300 Decompose (Reddish brown)	81	53.84 (53.17)	3.88 (3.53)	6.16 (5.17)	11.08 (10.30)	13.95 (13.08)	9
$C_{24}H_{21}ClN_2O_6Zn$ (534.28)	> 300 (Pink-Orange)	74	54.32 (53.95)	4.02 (3.96)	6.05 (5.24)	12.95 (12.24)	7.05 (6.64)	9
$C_{24}H_{23}N_3O_7Mo$ (561.40)	267 - 270 (Dark grey)	69	52.07 (51.35)	3.67 (4.13)	8.39 (7.48)	16.66 (17.09)	–	64
$C_{24}H_{17}ClN_2O_4Cd$ (545.27)	258 - 260 (Reddish brown)	84	53.66 (52.87)	3.71 (3.14)	5.93 (5.14)	21.08 (20.62)	6.91 (6.50)	20

NMR analysis

In the ^{1}H-NMR spectrum for the ligand, the hydroxyl proton is observed as a singlet at 9.16 ppm (Figure 1). In comparison, eugenol exhibits an OH resonance at 5.0-6.0 ppm [18]. The downfield shift of the hydroxyl proton to 9.16 ppm indicates a deshielded proton environment, which may result from the combined influence of the local electronic environment, substituent effects, and other structural features within the azo ligand framework [19]. At 6.49-8.58 ppm, aromatic protons display as multiplets, doublets, and a singlet. Vinyl protons resonate at 6.34-6.38 ppm, while the methoxy (Ar-OCH_3) group produces a singlet at 2.69 ppm [20]. The ^{13}C-NMR spectrum of the ligand displays signals at 186.85 and 195.31 ppm, corresponding to carbonyl carbons in the anthraquinone moiety. The resonance observed at 180.30 ppm may indicate the presence of the keto tautomeric form [21, 22] (Scheme 1).

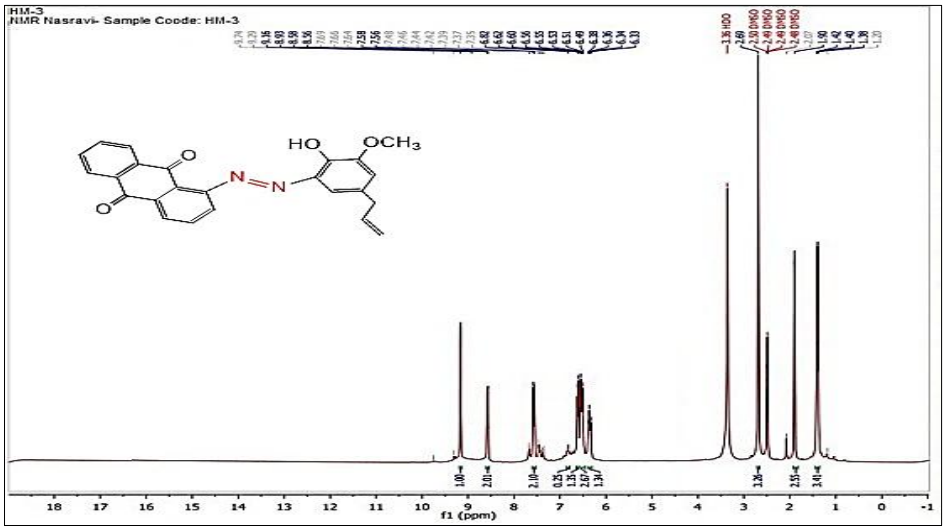
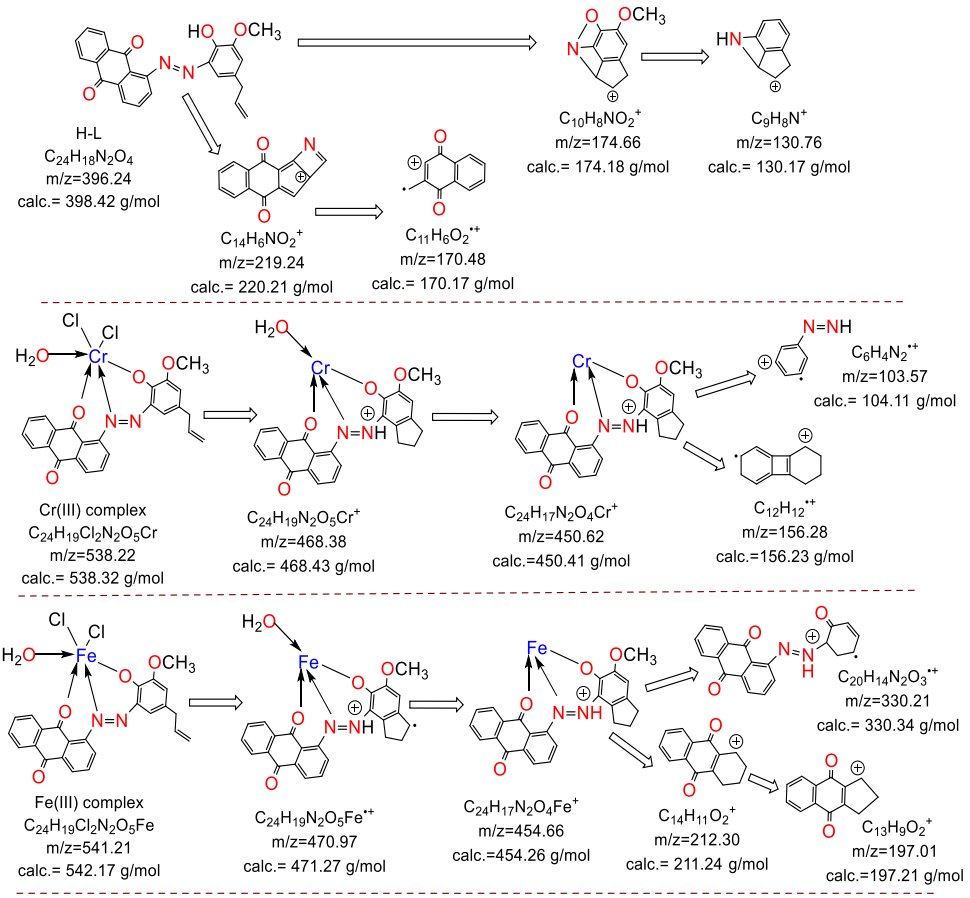


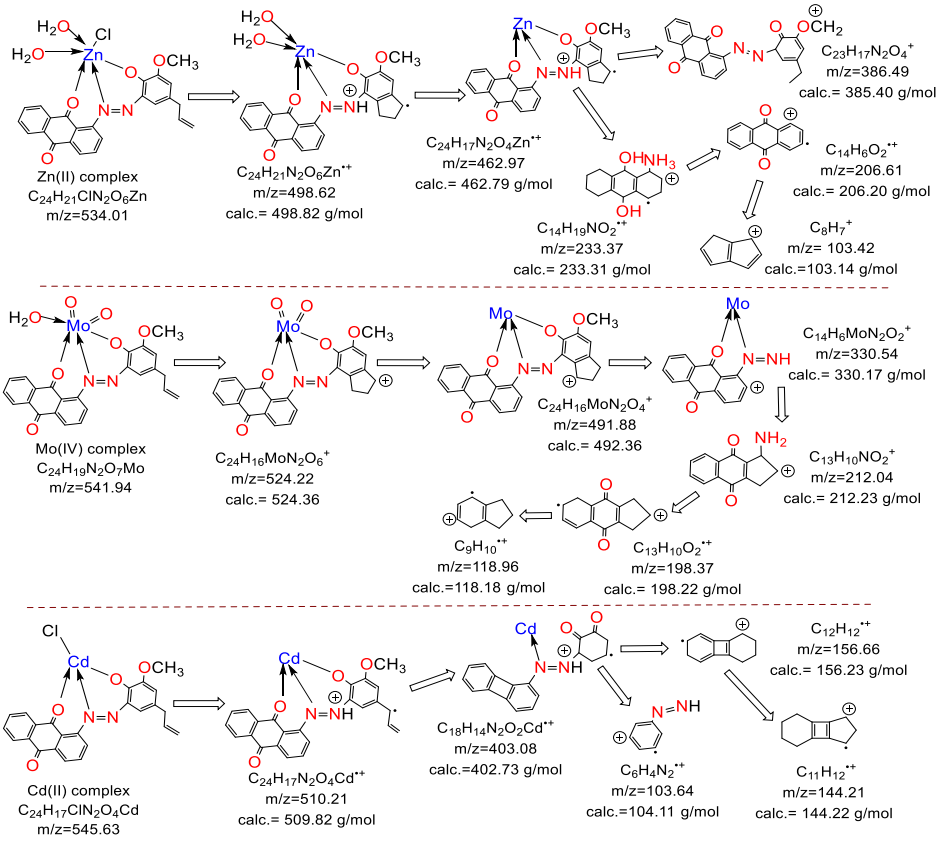
Figure 1. ^{1}H-NMR spectrum of H-L ligand (DMSO-d_6).

Mass spectrometric studies

Mass spectrometric analysis provided apparent corroboration of the proposed structures of the ligand and its metal complexes. The free azo H-L ligand exhibited an ion molecular peak at m/z 396.24, in excellent agreement with its calculated molecular weight (398.42 g mol^{-1}, $C_{24}H_{18}N_2O_4$). At the same time, the observed fragmentation pattern further validated the structural assignment. The spectra for the chelated states of Cr(III), Fe(III), Zn(II), and Cd(II) display molecular ion

peaks at m/z 538.22, 541.21, 534.01, and 545.63, respectively. In the Mo(IV) complex, an ion peak at m/z 541.94 is observed, which may be assigned to the molecular species formed after loss of the outer-sphere ammonium ion during ionization. These peaks are consistent with their respective formula weights and distinctive fragment ions, supporting the proposed molecular compositions of the compounds. The mass fragmentation patterns [23] of the free azo H-L ligand, as well as the proposed structures related to the complexes, as illustrated in Scheme 2, support their suggested formulas.





Scheme 2. The proposed mass fragmentation pattern of the free ligand with its complexes.

IR spectral studies

The coordination mode can be identified by comparing the infrared spectra of the free ligand and its metal complexes, as indicated in Table 2. The shift of the phenolic C-O stretching band from 1115 cm-1 in the free ligand to approximately 1170 cm^{-1} in the chelates suggests coordination through the phenolic oxygen atom and increased electron-density delocalization toward the metal center, thereby modifying the C-O bond environment [24]. Compared to the free ligand (1452 and 1469 cm^{-1}), the ν(N=N) stretching band in the complexes shifts to a higher frequency, confirming that the azo group coordinates across the nitrogen atom [25]. The bands near 1589 cm^{-1} remain unchanged and are attributed to aromatic ν(C=C) vibrations [23]. A conjugation of the carbonyl groups with the azo linkage reduces the ν(C=O) stretching frequency to 1635 cm^{-1} [26]. When coordinating with the metal ions, the carbonyl absorption shifts to (1622-1625) cm^{-1} in all complexes, supporting the coordination of the carbonyl oxygen to the metal center. The formation of a chelate ring in all complexes is supported by the appearance of a new band at (1541-1546 cm^{-1}) assigned to ring vibration. The infrared spectrum of the Mo(IV) complex reveals a stretching peak for Mo=O at 1049 cm^{-1} [27], and a broad N-H band is marked around 3200 cm^{-1}, which is characteristic of a free NH_4^+ ion [28]. Thermogravimetric analysis further confirmed the presence of coordinated water in the complexes, corresponding to $\rho_r(H_2O)$ and $\rho_w(H_2O)$ [29]. According to these results, the ligand functions as a tridentate chelator.

Table 2. The main indication of the infrared bands in the compounds (cm^{-1}).

Compounds	ν(OH)phenolic	ν(N=N)	ν(chelate ring)	ν(C=O) Coor.	ν(H_2O)aqua	ν(M-O) ν(M-N) ν(M-Cl)
	ν(C-O) phenolic			ν(C=O) Uncoor.	ρr ρw	
H-L	3421	1469	–	1635	–	–
	1115	1452		1676		
[Cr(L)(H_2O) Cl_2]	–	1510	1546	1624	3433	594
	1168	1473		1676	879	522
					771	351
[Fe(L)(H_2O) Cl_2]	–	1504	1546	1622	3438	551
	1168	1473		1676	889	435
					657	358
[Zn(L)(H_2O)$_2$Cl]	–	1510	1542	1625	3433	503
	1137	1452		1672	804	437
					673	349
NH_4[Mo(L)(H_2O) O_2]	–	1508	1541	1623	3446	594
	1170	1471		1677	879	524
					659	–
[Cd(L)Cl]	–	1508	1544	1623	–	530
	1168	1473		1676		470
						331

UV-visible spectra and magnetic data

The UV-Vis spectra of the H-L azo ligand and its chelates were examined in DMSO over a range of 200-1100 nm (Table 3). The free azo H-L ligand exhibits two significant absorption bands: one corresponding to a $\pi \rightarrow \pi^*$ transition at 34482 cm^{-1} and the other corresponding to an $n \rightarrow \pi^*$ transition at 27322 cm^{-1} [30]. Additionally, a lower-energy band at 22883 cm^{-1} is tentatively assigned to intramolecular charge transfer (ICT) associated with the conjugated azo-aromatic system [31]. The Cr(III) complex displayed two absorption bands at 15898 and 21276 cm^{-1}. These bands correspond to the transitions $^4A_2g \rightarrow {}^4T_2g(F)$ (ν_1) and $^4A_2g \rightarrow {}^4T_1g(F)$ (ν_2). According to the d^3 (Tanabe-Sugano) diagram, the resulting ratio of Dq/B' (3.0) compared to ν_2/ν_1(1.34) indicates that the Cr(III) ion is in an octahedral environment [32]. These transitions, along with a magnetic moment of 3.84 B.M. [33], agree with the expected values for Cr(III) cations that possess octahedral geometries and three unpaired electrons. The electronic spectrum of the Fe(III) chelate displayed two ligand-centered absorption bands along with two lower-energy bands at 19157 cm^{-1} and 18975 cm^{-1}, which are assigned to charge transfer transitions and spin-forbidden ^6A_1g to ^4A_1g and ^4Eg (G) transitions, respectively, which suggest an octahedral structure, supported by a magnetic moment value of 5.78 B.M. [34]. The Mo(IV) complex is proposed to adopt an octahedral structure, supported by its magnetic moment value of 2.64 B.M. and a broad absorption band at 18587 cm^{-1}, characteristic of the $^3T_1g(F) \rightarrow {}^3T_2g$ transition [27]. Both Zn(II) and Cd(II) complexes exhibit only charge transfer bands at 21645 and 20746 cm^{-1}, respectively, and are both diamagnetic (d^{10}) [35]. Elemental analysis, mass spectrometry, and thermal data supported that Zn(II) forms a six-coordinate (octahedral) complex with a multidentate ligand [36]. In contrast, Cd(II), being larger in size, typically adopts four-coordinate (tetrahedral) geometries under similar ligand and steric conditions [37].

Table 3. The electronic spectral and magnetic details of the free H-L and chelated.

Compounds	λ (nm)	Absorption bands (cm^{-1})	Transition Character	μ_{eff} /B.M. (Found) Calc.	Proposed Geometry
H-L	290 366 437	34482 27322 22883	π→π* n→π* ICT	–	–
$[Cr(L)(H_2O)\ Cl_2]$	294 324 470 629	34013 30864 21276 15898	π→π* n→π* $^4A_2g \rightarrow {}^4T_1g\ (F)$ $^4A_2g \rightarrow {}^4T_2g\ (F)$	(3.84) 3.87	Octahedral
$[Fe(L)(H_2O)\ Cl_2]$	289 325 394 527	34602 30769 19157 18975	π→π* n→π* C.T $^6A_1g \rightarrow {}^4A_1g,\ {}^4Eg\ (G)$	(5.78) 5.92	Octahedral
$[Zn(L)(H_2O)_2Cl]$	282 338 462	35460 29585 21645	π→π* n→π* C.T	Diam	Octahedral
$NH_4[Mo(L)(H_2O)O_2]$	282 323 538	35460 30959 18587	π→π* n→π* $^3T_1g\ (F) \rightarrow {}^3T_2g$	(2.64) 2.83	Octahedral
[Cd(L)Cl]	292 320 482	34246 31250 20746	π→π* n→π* C.T	Diam	Tetrahedral

Diam= Diamagnetic

Thermal analysis

The H-L ligand undergoes thermal decomposition in four stages, as indicated by thermogravimetric (TG) analysis. Zn(II), Mo(IV), and Cd(II) complexes decompose in two phases, while Cr(III) and Fe(III) complexes decompose in three phases. According to the TG data, all complexes except the Cd(II) complex contain coordinated water. During the initial stages of heating, the coordinated water molecules are eliminated. Additionally, the Mo(IV) complex exhibits a low-temperature event at 32.00 °C, likely due to a slight reduction, phase transition, or decomposition [24]. The good agreement between the experimental and theoretical mass losses and residual weights supports the proposed thermal degradation pathways [38, 39]. The relevant findings of TG and DSC performed in a nitrogen atmosphere are listed in Tables 4 and 5, respectively.

Table 4. TGA data for H-L ligand and metal complexes.

Compounds (M.Wt) g./mol	TG Temp. range (°C)	Wt. loss (%) (Found) Calc.	Lost species	Residuals (%) (Found) Calc.
H-L (398.42)	109.23-263.97	(35.330) 35.389	$C_4H_5 + 2CO_2$	2C (6.360) 6.028
	279.81-325.66	(17.560) 17.318	C_5H_9	
	327.77-534.95	(21.530) 22.087	C_6H_2N	
	534.99-597.88	(19.220) 19.075	C_5H_2N	

[Cr-complex] (538.32)	40.22-116.39	(6.750) 6.594	Cl	Cr+5C (20.250) 20.867
	161.58-339.85	(33.276) 32.972	H_2O + HCl + $C_6H_5NO_2$	
	345.47-547.78	(39.720) 39.567	$C_{13}H_{11}NO_2$	
[Fe-complex] (542.17)	56.48-185.27	(22.509) 22.317	2HCl + H_2O + C_2H_6	Fe + 6C (24.361) 23.642
	196.78-386.79	(45.552) 45.926	$C_{14}H_5N_2O_3$	
	393.38-569.74	(7.578) 8.115	C_3H_4O	
[Zn-complex] (534.28)	65.58-263.47	(41.498) 41.083	2H_2O + Cl + $C_8H_8N_2O$	ZnO + 5C (25.813) 26.461
	273.47-557.48	(32.689) 32.380	$C_{11}H_9O_2$	
[Mo-complex] (561.40)	52.48-301.59	(43.249) 43.284	H_2O + NH_3 + $C_{13}H_8N_2O$	MoO_2 + 2C (27.18) 27.066
	308.76-556.82	(29.570) 29.568	$C_9H_{10}N_2O_3$	
[Cd-complex] (545.27)	197.80-395.60	(55.752) 54.743	$C_{16}H_{11}N_2O_2Cl$	Cd (19.08) 20.613
	402.54-571.13	(25.161) 24.574	$C_8H_6O_2$	

Table 5. The data analysis of DSC for H-L ligand and metal complexes.

Compounds (M.Wt) g./mol	T_i - T_f (°C)	T_{max} (°C)	ΔH (J/g)	ΔS (J/mol. K)	ΔG_{298} (J/mol)
H-L(398.42)	155.36-159.31	157.53	-0.39	-0.35	-46.73
	313.14-319.44	315.37	-3.29	-2.22	-646.73
[Cr-complex] (538.32)	42.08-109.67	65.77	-40.82	-64.83	-2643.36
	124.69-175.67	150.72	-20.43	-25.94	-3261.97
	184.39-198.69	192.37	-4.86	4.86	-940.62
	219.38-307.69	256.07	-2.97	-3.02	-698.07
[Fe-complex] (542.17)	68.28-128.63	96.89	-89.67	-131.38	-9445.01
[Zn-complex] (534.28)	21.38-113.25	78.88	208.64	316.65	17061.38
	221.08-358.67	287.64	248.61	236.85	62208.26
[Mo-complex] (561.40)	26.38-43.22	32	-2.41	-4.43	-31.03
	43.22-84.47	65.63	-37.06	-61.41	-2495.20
	167.35-248.37	216.84	-98.67	-113.04	-21687.49
	314.75-321.08	318.24	-3.72	-3.53	-1035.53
[Cd-complex] (545.27)	23.67-109.63	76.98	209.37	326.05	16948.56
	217.68-357.84	293.47	317	305.05	81898.27

In-vitro biological evaluation

Antioxidant activity

The efficiency of the azo H-L ligand and its metal chelates to scavenge free radicals was assessed in vitro using gallic acid and Trolox as reference standards. The compounds demonstrated lower activity than gallic acid; however, the ligand (H-L) and Cr(III), Fe(III), and Mo(IV) complexes exhibited notable scavenging efficiencies, with the Cr(III) complex showing potency comparable to that of Trolox. The inhibitory effects on DPPH radicals were concentration-dependent, with suppression ratios increasing with sample concentration (Figure 2). In contrast, the Zn(II) and Cd(II) complexes exhibited lower activities, at around 76%. The antioxidant activity was evaluated using IC_{50} values (µg/mL), yielding the following results: gallic acid (21.58 ± 0.54) > azo H-L ligand (36.89 ± 0.41) ~ Fe(III) (39.87 ± 0.22), Mo(IV) (39.42 ± 0.38) complexes > Cr(III) complex (41.97 ± 0.32) > Cd (II) complex (44.14 ± 0.59) > Zn(II) complex (48.33 ± 0.52). The potency of the Cr(III) complex (IC_{50} = 41.97 ± 0.32 µg/mL) was comparable to that of Trolox (IC_{50} = 41.66 ± 0.29 µg/mL), with no statistically significant difference observed ($t(6) = 1.44$, $p = 0.20$; $n = 4$). The differences in DPPH radical-scavenging activity might be attributed to the characteristics of the central metal ion and its coordination environment. The redox properties, which depend on the metal, along with variations in electronic characteristics and the availability of ligand donor groups upon coordination, may influence the electron-transfer ability of the complexes and, consequently, their antioxidant activity [40]. These results suggest that the H-L ligand and Fe(III), Mo(IV), and Cr(III) complexes possess promising in vitro antioxidant activity and require further biological investigation [26, 41, 42].

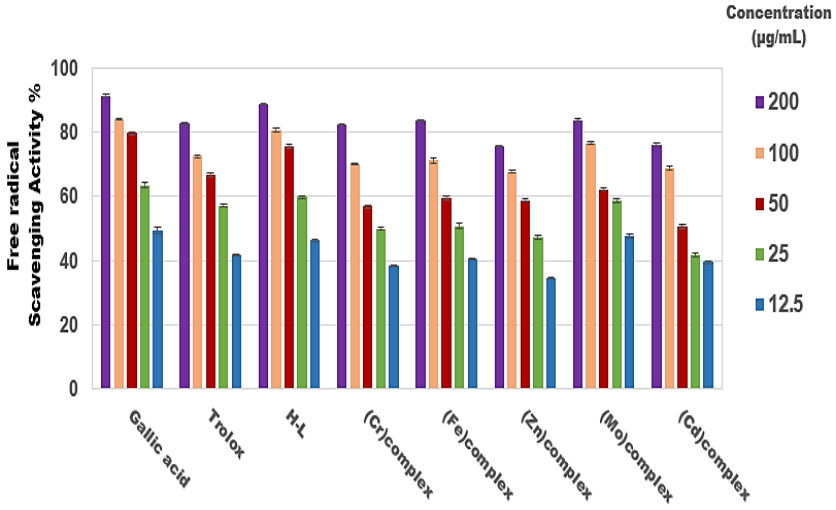


Figure 2. The *in vitro* DPPH scavenging potency of H-L and its complexes; mean ± SD, n = 4.

DNA cleavage studies

The DNA cleavage ability of the ligand with its metal chelates was evaluated employing DNA from *Proteus mirabilis* (a Gram-negative, Group P) and *Staphylococcus aureus* (a Gram-positive, Group S). The control lanes (C_1, C_2) revealed that the DNA remained unchanged, with no cleavage observed in the absence of any test compounds, as shown in Figure 3. In lanes P_1 and S_1, the free H-L ligand caused only a minimal decrease in DNA band intensity, showing relatively low DNA-

cleavage activity. The Cd(II) complex displayed no activity against either bacterial species. In contrast, the Fe(III), Zn(II), and Mo(IV) complexes exhibited comparable, albeit slight, effects on Gram-positive DNA. In comparison, the Cr(III), Fe(III), Zn(II), and Mo(IV) complexes (lanes P_2-P_5, S_2) produced pronounced alterations in the DNA bands, suggesting appreciable DNA-cleavage activity. Since no exogenous reductants or oxidants were employed, a hydrolytic pathway may contribute to the observed DNA cleavage [13]. These results show the importance of metal coordination in facilitating DNA separation, particularly when nitrogen and oxygen-donor atoms are involved [19]. According to numerous reports in the literature, some metal complexes and related compounds can induce DNA cleavage. The DNA-cleavage activity observed in the present study suggests that the tested compounds can interact with DNA and induce DNA cleavage under the experimental conditions [43].

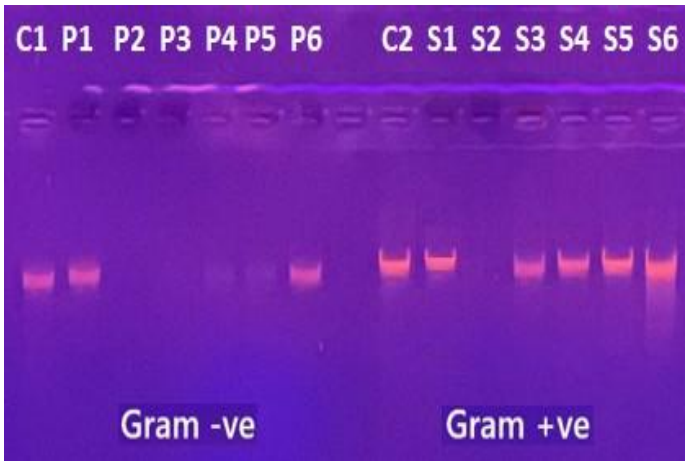


Figure 3. DNA cleavage (gel electrophoresis design) for the *P. mirabilis* (Group P) and *S. aureus* (Group S); C_1, C_2: Controls; Lanes P_1-P_6 / S_1-S_6: H-L, Cr, Fe, Zn, Mo and Cd complexes, respectively.

CONCLUSION

The anthraquinone nucleus and natural phenolic monoterpene, along with its metal complexes of Cr(III), Fe(III), Zn(II), Mo(IV), and Cd(II), were utilized to synthesize a tridentate azo (H-L) ligand 1-(5-allyl-2-hydroxy-3-methoxyphenyl)diazenyl)anthraquinone, which has been successfully synthesized and extensively investigated. Biological studies have demonstrated that the free ligand (H-L) and its Cr(III), Fe(III), and Mo(IV) complexes showed promising in vitro antioxidant activity, with variations in radical-scavenging efficiency that may be influenced by the metal ion and ligand architecture. The essential role of metal coordination in regulating biomolecular interactions was emphasized further by DNA cleavage studies, which revealed that all complexes, except for the Cd(II) complex, exhibited more pronounced DNA-cleavage activity than the free ligand, without the use of any external reductants or oxidants. These results highlight the versatility of anthraquinone-natural monoterpene (eugenol)-based azo ligand as a scaffold for designing coordination compounds with potential redox properties and DNA-cleavage activity. Additionally, these findings provide insights into the potential catalytic and biological properties of functional metal-based compounds.

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