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Manganese(II) Complexes of Biological Relevance: Synthesis and Spectroscopic Characterization of Novel Manganese(II) Complexes with Monobasic Bidentate Ligands Derived from Halo-Substituted 1*H*-Indole-2,3-diones[1]

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Abstract—Novel biologically significant manganese(II) complexes with four monobasic bidentate ligands L^1H [2-(5-fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarboxamide], L^2H [2-(5-fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarbothioamide], L^3H [2-(5-bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarboxamide] and L^4H [2-(5-bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarbothioamide] were synthesized by complexation of the ligands with $MnCl_2 \cdot 4H_2O$ in 1 : 1 and 1 : 2 molar ratios in methanol. The Schiff base ligands and complexes were characterized by elemental analyses, melting points, molecular weights, IR, ^1H and ^{13}C NMR, UV–Vis, EPR, and mass spectra, as well as X-ray powder diffraction patterns. Based on the spectral data, a tetrahedral geometry was proposed for all the synthesized metal complexes. The ligands and complexes were tested *in vitro* against bacteria (*Escherichia coli* and *Staphylococcus aureus*) and fungi (*Fusarium semitectum* and *Aspergillus flavus*) to show that they were active against all the microbial strains examined, and the metal complexes were more active in comparison with the ligands. DNA cleavage activity of the complexes was examined by gel electrophoresis.

Keywords: Schiff bases, metal complexes, spectroscopy, antimicrobial activity, DNA cleavage activity

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INTRODUCTION

Schiff bases have been extensively studied as chelating agents for transition metals due to their extraordinary synthetic flexibility, selectivity, and sensitivity to the central metal atom [1–8]. Isatin and its derivatives occupy a unique place in the Schiff base family and have broad-range applications, as they show CNS depressant, cytotoxic, anti-HIV, anticonvulsant, analgesic, and many more other activities [9–13]. Isatin thiosemicarbazone derivatives possess a number of chemotherapeutic properties like ability to inhibit enzymes, as well as antimicrobial, antiulcer, and antiviral activities [14–17]. Additional donor sites, specifically >C=O, >C=N–, etc., in halo-substituted isatin Schiff bases, make the latter more flexible and versatile chelating agents [18–19].

Many authors reported that the biological activity of Schiff bases is much enhanced by their coordination with suitable metal ions [20–22]. The discovery of antimicrobial activity formed one of the most fundamental findings in medicinal history [23, 24]. In recent years, the interaction of transition metal complexes with DNA was broadly studied for their application in chemotherapy and as probes for DNA structure [25, 26]. It was found that some of the complexes are able to cleave DNA. A huge number of publications are available on the DNA binding studies of Schiff bases and their metal complexes [27–31].

Manganese is a biologically relevant transition metal playing an important role in biotechnology, biomedical nanotechnology, biomimicking, and chemotherapeutic approaches [32, 33]. The coordination chemistry of manganese complexes with nitrogen or sulfur donor moieties is of great interest as these complexes give information about the functional role of manganese(II)

[1] The text was submitted by the authors in English.

in biological systems and possible magnetic coupling interactions. Manganese complexes of Schiff bases have found wide application in food and dye industry, analytical chemistry, catalysis, and as fungicidal, flame-retardant, and antiradical agents [34–36].

Encouraged by the above findings and our interest in the biological and chemical properties of such compounds, we set ourselves the task to design and synthesize some new isatin derived Schiff bases and explore their coordination behavior toward the manganese metal ion. The ligands and some selected manganese complexes have also been screened to assess their antimicrobial and DNA cleavage activities.

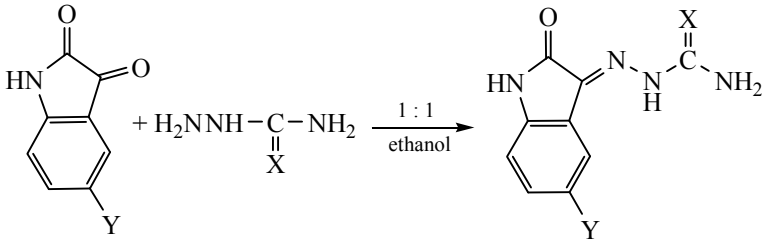
RESULTS AND DISCUSSION

Schiff base ligands L^1H [2-(5-fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene)-hydrazinecarboxamide], L^2H [2-(5-fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarbothioamide], L^3H [2-(5-bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarboxamide], and L^4H [2-(5-bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarbothioamide] were synthesized by the reaction of 5-flouroisatin (L^1H, L^2H) and 5-bromoisatin (L^3H, L^4H) with hydrazinecarboxamide (in the presence of sodium acetate) and hydrazinecarbothioamide in a 1 : 1 molar ratio in absolute ethanol, respectively [reaction (1)]. The tautomeric forms of the ligands are shown in equilibrium (2).

The formation of manganese complexes of ligands LH is presented by reactions (3) and (4).

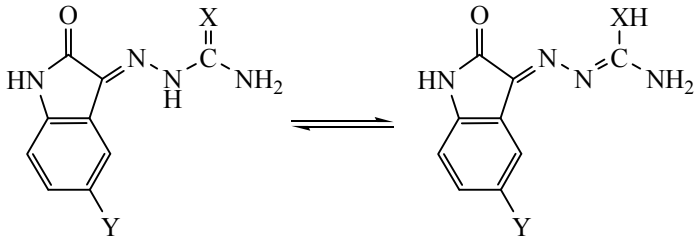
The spectral data and elemental analyses of the products are consistent with the general formulas $[MnCl(L)(H_2O)]$ and $[(Mn(L)_2]$. The synthesized manganese complexes are colored solids soluble in methanol, DMF, and DMSO. They have a monomeric nature as indicated by molecular weight determination.

Coordination produces changes in the IR spectra of the ligands. The band at 1600–1615 cm^{-1} attributed to the >C=N group in the IR spectra of the free ligands shifts to lower wavenumbers in the spectra of the complexes, which suggests coordination of the azomethine nitrogen to Mn. The IR spectra of the free ligands display two sharp bands at 3440–3495 and 3320–3350 cm^{-1} due to the ν_{as} and ν_s modes of the NH_2 group, respectively. These bands remain at almost the same positions in the spectra of the complexes, thereby suggesting that NH_2 is not involved in chelation. The same relates to the 1*H*-indole-2,3-dione moiety: the



(1)

X = O, Y = F (L^1H), Br (L^3H); X=S, Y=F (L^2H), Br (L^4H).



(2)

$$MnCl_2 \cdot 4H_2O + LH \xrightarrow[\text{methanol}]{1:1} [MnCl(L)(H_2O)] + HCl + 3H_2O, \quad (3)$$

$[MnCl(L)(H_2O)]$: L = L^1 (**1**), L^2 (**2**), L^3 (**3**), L^4 (**4**).

$$MnCl_2 \cdot 4H_2O + 2LH \xrightarrow[\text{methanol}]{1:2} [Mn(L)_2] + 2HCl + 4H_2O, \quad (4)$$

$[Mn(L)_2]$: L = L^1 (**5**), L^2 (**6**), L^3 (**7**), L^4 (**8**).

Table 1. X-ray powder diffraction data for $[MnCl(L^4)(H_2O)]$ complex **4**

h	*k*	*l*	$2\theta_{exp}$, deg	$2\theta_{calc}$, deg	d_{exp}, Å	d_{calc}, Å	I_{rel}, %	$2\theta_{exp} - 2\theta_{calc}$, deg
1	0	2	15.7188	16.093	5.63785	5.50322	53.6	–0.3742
0	2	0	18.6656	18.598	4.75393	4.76700	45.05	0.0676
1	2	1	20.2101	20.213	4.39396	4.18493	28.45	–0.0029
4	0	0	28.7969	28.715	3.10032	3.11275	10.95	0.0819
1	1	5	38.3237	38.349	2.34872	2.33357	16.37	–0.0253
4	1	4	42.5395	42.465	2.1252	2.12698	7.88	0.0745
6	1	0	44.6151	44.654	2.03104	2.02769	100	–0.0389

strong indole ν(NH) band remains at almost the same position in the spectra of the ligands and complexes (3170–3180 cm^{-1}). Comparative analysis of the IR spectra of the complexes and ligands shows the disappearance of the broad band at 3265–3286 cm^{-1} due to the ν(NH) mode of semicarbazone and thiosemicarbazone moiety provides evidence for the deprotonation of this group on complexation. The ν(C=S) or ν(C=O) bands of thiosemicarbazone or semicarbazone moiety at, respectively, 1050–1065 and 1685–1696 cm^{-1} in the IR spectra of the ligands are shifted to lower wavenumbers in IR spectra of the complexes, implying involvement of these thiolic sulfur or carbonyl oxygen in coordination with the metal ion. The band at 1720 cm^{-1} due to the isatin C=O bond remains unchanged in the complexes, indicating that this C=O group is not involved in coordination. The bands at 465–480, 386–401, and 310–324 cm^{-1} can be attributed to the ν(Mn–O), ν(Mn–N), and ν(Mn–S) modes, respectively. The band at 800–880 cm^{-1} in the $Mn(L_2)$ complexes can be assigned to the rocking mode of the coordinated water molecule [37].

The EI mass spectrum of $[MnCl(L^4)(H_2O)]$ complex **4** was studied as a representative case. The molecular ion peak of the complex was observed at *m/z* 406.75, and this is in good agreement with its molecular weight, which suggests the monomeric nature of the complex and confirms the proposed formula.

The electronic absorption (EA) spectra of ligands LH and their complexes **1–8** were measured in DMSO and gave adequate support in establishing the geometry of the metal complexes. The expected tetrahedral geometry of the complexes is supported by the observation of very weak *d–d* absorption bands in the visible region at 16500–18000 and 19720–20500 cm^{-1} which can be assigned to the $^6A_1 \rightarrow {}^4T_2(\nu_1)$ and $^6A_1 \rightarrow {}^4E(\nu_2)$ spin-forbidden transitions [38]. The EA spectra of the ligands show a broad band at 26595–27027 cm^{-1} due to $n–\pi^*$ transitions of the azomethine group and undergo a blue shift on complexation, indicating that this group is involved in coordination with the metal ion. The bands at 36630–37174 and 31250–31545 nm due to $\pi–\pi^*$ transitions in the ligands does not change their positions in the EA spectra of the complexes.

The EPR spectra of complexes **1–8** were recorded at room and liquid nitrogen temperatures. The EPR spectrum of complex **4** at liquid nitrogen temperature consists of a single broad peak with the *g* factor estimated at 2.014. The estimated *g* factors for complexes **1–8** span the range 1.983–2.015, which is in accordance with the reported values for tetrahedral Mn(II) complexes [39].

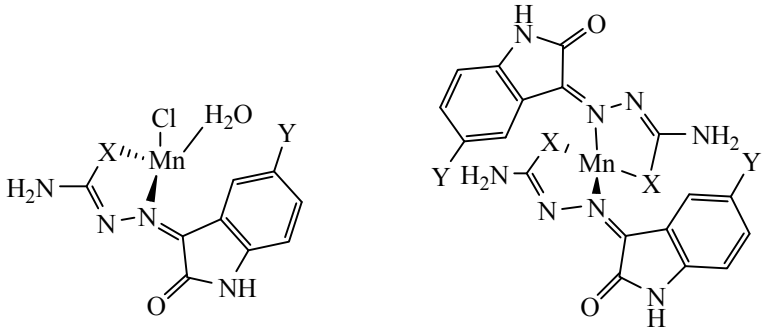
The magnetic moments of the synthesized complexes **1–8** were found to range from 5.77 to 5.95 B.M. Such values suggest a high-spin state of Mn(II) in the complexes, which is due to the presence of five unpaired electrons.

X-ray powder diffraction study of a representative powdered sample of complex **4** was performed to find out the lattice dynamics of the complexes. Interplanar spacings (*d*, Å) were measured, and the Miller indices *h*, *k*, *l* were assigned to each *d* value. The 2θ, *d*, and *hkl* values for complex **4** are listed in Table 1. The obtained X-ray diffraction data point to an orthorhombic unit cell with the following parameters: a = 12.451 Å, b = 9.5340 Å, c = 12.27 Å, $\alpha = \beta = \gamma = 90°$.

Based on the above physicochemical and spectral evidence, we can suggest that the newly synthesized manganese complexes **1–8** are tetrahedral and have the structures as described in Scheme 1.

We evaluated the biological activity of ligands $L^1H–L^4H$ and their Mn(II) complexes **1–8** against two

Scheme 1.



X = O/S, Y = F/Br.

bacteria (*Staphylococcus aureus* and *Escherichia coli*) and two fungi (*Fusarium semitectum* and *Aspergillus flavus*). The summarized data (shown in Table 2 and Table 3) were compared with those for the standard drug Streptomycin (for bacteria) and Itraconazole (for fungi). All the fungal and bacterial strains tested proved to be sensitive both to the ligands and to their respective manganese complexes. The results clearly revealed a significant increase in toxicity of the complexes as compared to their respective ligands. The enhancement of the biological activity of the complexes can be explained by the fact that complexes contain Mn, while ligands not.

DNA is the primary target molecule for most anticancer and antiviral therapies according to cell biology. When some kinds of metal complexes interact with DNA, they may induce breakage of DNA strands. The nature of the ligand and metals is of main importance in the interaction of the complexes with the DNA molecule, which would help in the design of newer drugs and develop new selective, efficient DNA recognition and cleaving agents [40]. The electrophoresis method was employed to study the efficiency of DNA cleavage by the synthesized ligands and Mn(II) complexes. The DNA cleavage activity was tested against *E. coli* (ATCC 25922) with the synthesized

Table 2. Antibacterial screening data of the ligands and their Mn(II) complexes

Compound	Diameter (mm) of inhibition zone after 24 h (conc. in ppm)			
	S. aureus		*E.coli*	
	500	1000	500	1000
L^1H	10	11	11	13
L^2H	11	13	12	14
L^3H	9	10	11	12
L^4H	10	11	12	13
$[Mn(L^1)Cl(H_2O)]$ (**1**)	13	15	14	16
$[Mn(L^2)Cl(H_2O)]$ (**2**)	15	17	17	19
$[Mn(L^3)Cl(H_2O)]$ (**3**)	12.5	14	15.5	17
$[Mn(L^4)Cl(H_2O)]$ (**4**)	14	16	16	18
$[Mn(L^1)_2)]$ (**5**)	14	16	16	19
$[Mn(L^2)_2]$ (**6**)	16	19	18	20
$[Mn(L^3)_2)]$ (**7**)	13	15	16	18
$[Mn(L^4)_2)]$ (**8**)	15	18	17	19
Streptomycin	26	30	28	34

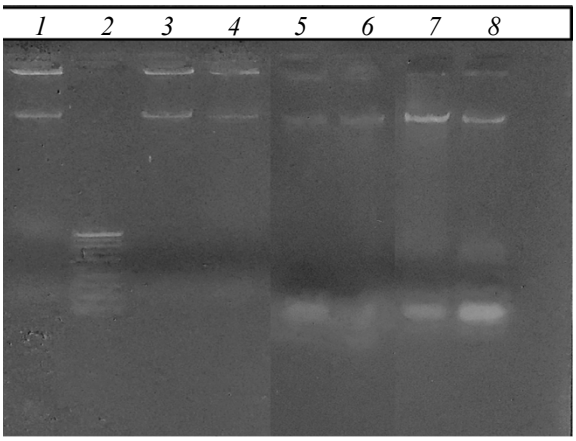
Table 3. Antifungal screening data for the ligands and their Mn(II) complexes

Compound	% Inhibition after 96 h (conc. in ppm)					
	Fusarium semitectum			*Aspergillus flavus*		
	50	100	200	50	100	200
L^1H	42	45	51	45	49	51
L^2H	45	50	53	47	52	55
L^3H	38	45	49	42	46	50
L^4H	44	46	52	45	47	53
$[Mn(L^1)Cl(H_2O)]$ (**1**)	51	54	60	52	56	60
$[Mn(L^2)Cl(H_2O)]$ (**2**)	56	59	65	57	62	66
$[Mn(L^3)Cl(H_2O)]$ (**3**)	49	53	57	50	54	59
$[Mn(L^4)Cl(H_2O)]$ (**4**)	54	57	63	55	61	64
$[Mn(L^1)_2)]$ (**5**)	53	56	62	55	59	63
$[Mn(L^2)_2]$ (**6**)	59	63	66	60	65	69
$[Mn(L^3)_2)]$ (**7**)	50	55	60	54	58	62
$[Mn(L^4)_2)]$ (**8**)	55	62	67	57	63	68
Itraconozole	93	100	100	86	100	100

ligands L^1H and L^2H and their complexes **1, 2, 5,** and **6** by agarose gel electrophoresis. Both the ligands and all the metal complexes showed DNA cleavage activity, as evidenced by the difference observed in the banding patterns of lanes *3–8* compared with the control DNA of *E. coli* (lane *1*). Untreated DNA of standard *E. coli* did not exhibit any cleavage (lane *1*). All the metal complexes showed better DNA cleavage activity than the free ligands. Thiosemicarbazone complexes **2** and **6** exhibit better DNA cleavage activity than semicarbazone. Thiosemicarbazones are better inhibitors of the enzyme ribonucleotide reductase and can interrupt DNA synthesis and repair. Coordination of metal with these thiosemicarbazone ligands results in enhancement of their biological activity [41]. The results of the isolated DNA cleavage assay display a significant role of the halo-substituted isatin ligand coordinated to the metal ion in DMSO (see figure).

EXPERIMENTAL

All chemicals (reagent grade) and solvents (analytical grade) were distilled over appropriate drying agents immediately prior to use. 5-Fluroindole-2,3-dione, 5-bromo-indole-2,3-dione, and $MnCl_2 \cdot 4H_2O$ were procured from Sigma-Aldrich. The molecular weights were determined by the Rast's camphor method [42]. Sulfur and nitrogen were estimated by the Messenger's [43] and Kjeldahl's [44] methods, respectively. Analyses for carbon and hydrogen were performed at the IIT Mumbai. Manganese was estimated gravimetrically. The melting points were



DNA cleavage activity of ligands L^1H and L^2H and their complexes **1**, **2**, **5, 6**. (*1*) DNA control; (*2*) DNA ladder; (*3*) DNA + L^1H; (*4*) DNA + L^2H; (*5*) DNA + **1**; (*6*) DNA + **5**; (*7*) DNA + **2**; and (*8*) DNA + **6**.

determined on an electrical melting point apparatus. Magnetic moment measurements were performed at the RSIC IIT (Chennai) on a PAR 155 vibrating sample magnetometer. The IR were recorded on a Nicolet Megna FTIR-550 spectrophotometer in KBr pellets. The ^{13}C NMR spectra were recorded at the MNIT (Jaipur) on a JEOL ECS 400 MHz NMR spectrometer in DMSO-d6 using TMS as internal standard. The ^{1}H NMR spectra were recorded at Thera Chem (Sitapura, Jaipur) on a Bruker-300 MHz NMR spectrometer in DMSO-d_6 using TMS as internal standard. The EPR spectra were monitored at the IIT (Mumbai) on a Varian E-line Century X-band EPR spectrometer (Model-E-112). The EA spectra were obtained on a Varian–Cary/5E spectrophotometer in DMSO. The powder X-ray diffraction measurements were performed at the MNIT (Jaipur) on a Panalytical Xpert Pro 3040 instrument (wave length = 1.54 Å).

2-(5-Fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene)-hydrazinecarboxamide (L^1H). An ethanolic solution (25 cm^3) of 5-flouroindole-2,3-dione (2 g, 12.11 mmol) was added to an ethanolic solution (25 cm^3) of semicarbazide hydrochloride (1.35 g, 12.11 mmol) in the presence of sodium acetate (1.055g, 12.11 mmol) in a 1 : 1 molar ratio. This reaction mixture was then refluxed on a water bath for 3–4 h and allowed to stand overnight. The ligand that precipitated was separated and purified by recrystallization from the same solvent (ethanol). Yield 1.88 g (70%), dark yellow crystals. mp 254–255°C. IR spectrum, ν, cm^{-1}: 3270 (NH), 1611 (C=N). ^{1}H NMR spectrum, δ, ppm: 12.32 s (1H, ring NH), 11.25 s (1H, free NH), 3.24 s (2H, NH_2), 6.31-8.36 m (aromatic protons). ^{13}C NMR spectrum, δ, ppm: 157.58 (azomethine carbon), 163.25 (enolic carbon), 107.66–155.57 (aromatic carbon). EA spectrum, λ_{max}, cm^{-1}: 26950 ($n \rightarrow \pi*$), 36870, 31400 ($\pi \rightarrow \pi*$). Found, %: C 48.34, H 3.11, N 24.76. $C_9H_7N_4O_2F$. Calculated, %: C 48.60, H 3.17, N 25.30. *M* 221.41 (calculated 222.37).

2-(5-Fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene)-hydrazinecarbothioamide (L^2H) was obtained by the same procedure from 5-flouroindole-2,3-dione (2 g, 12.11 mmol) and thiosemicarbazide (1.10 g, 12.11 mmol), except that no sodium acetate was added. Yield 1.98 g (69%), dark orange crystals. mp 275–276°C. IR spectrum, ν, cm^{-1}: 3286 (NH), 1615 (C=N). ^{1}H NMR spectrum, δ, ppm: 12.62 s (1H, ring NH), 11.38 s (1H, free NH), 3.36 s (2H, NH_2), 7.10–9.14 m (aromatic protons). ^{13}C NMR spectrum, δ, ppm: 161.98 (azomethine carbon), 178.53 (thiolic carbon), 108.58–157.10 (aromatic carbon). EA spectrum, λ_{max}, cm^{-1}: 27027 ($n \rightarrow \pi*$), 37174, 31545 ($\pi \rightarrow \pi*$). Found, %: C 45.02, H 2.49, N 23.13, S 13.01. $C_9H_7N_4OFS$. Calculated, %: C 45.33, H 2.95, N 23.59, S 13.44. *M* 237.22 (calculated 238.45).

2-(5-Bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)-hydrazinecarboxamide (L^3H) was prepared as described for ligand L^1H from 5-bromoindole-2,3-dione (2 g, 8.84 mmol), semicarbazide hydrochloride (0.98 g, 8.84 mmol), and sodium acetate (0.73 g, 8.84 mmol)]. Yield 1.80 g (72%), light yellow crystals. mp 279–280°C. IR spectrum, ν, cm^{-1}: 3265 (NH), 1600 (C=N). ^{1}H NMR spectrum, δ, ppm: 11.01 s (1H, ring NH), 10.47 s (1H, free NH), 3.14 s (2H, NH_2), 6.17-8.29 m (aromatic protons). ^{13}C NMR spectrum, δ, ppm: 156.45 (azomethine carbon), 162.92 (enolic carbon), 112.78-142.12 (aromatic carbon). EA spectrum, λ_{max}, cm^{-1}: 26595 ($n \rightarrow \pi*$), 36630, 31250 ($\pi \rightarrow \pi*$). Found, %: C 37.89, H 2.33, N 19.34. $C_9H_7N_4O_2Br$. Calculated, %: C 38.15, H 2.49, N 19.85. *M* 282.12 (calculated 283.30).

2-(5-Bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)-hydrazinecarbothioamide (L^4H) was prepared as described for ligand L^2H from 5-bromoindole-2,3-dione (2 g, 8.84 mmol) and thiosemicarbazide (0.80 g, 8.84 mmol)]. Yield 1.98 g (75%), reddish brown crystals. mp 264–265°C. IR spectrum, ν, cm^{-1}: 3271 (NH), 1610 (C=N). ^{1}H NMR spectrum, δ, ppm: 12.35 s (1H, ring NH), 11.27 s (1H, free NH), 3.73 s (2H, NH_2), 6.88–8.45 m (aromatic protons). ^{13}C NMR spectrum, δ, ppm: 157.55 (azomethine carbon), 176.15 (thiolic carbon), 112.54–141.33 (aromatic carbon). EA spectrum, λ_{max}, cm^{-1}: 26700 ($n \rightarrow \pi*$), 36750, 31365 ($\pi \rightarrow \pi*$). Found, %: C 35.99, H 2.20, N 18.71, S 10.99. $C_9H_7N_4O_2SBr$. Calculated, %: C 36.10, H 2.35, N 18.79, S 10.70. *M* 298.77 (calculated 299.37).

Synthesis of Mn(II) complexes 1–8 *(general procedure).* A solution of $MnCl_2 \cdot 4H_2O$ (4.54 mmol) in methanol (25 cm^3) was added to a solution of ligand LH (4.54 g, 9.08 mmol) in methanol (25 cm^3) in a 1 : 1 or 1 : 2 M : L molar ratio. The resulting mixture was heated under reflux for 14–15 h on glass apparatus fitted with quickfit interchangeable standard ground joints. After the reaction was complete, the solvent was removed under reduced pressure, and the solid product was washed with cyclohexane and recrystallized from methanol.

Aquochloro[2-(5-fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene) hydrazinecarboxamide]manganese(II) (1). Yield 1.06 g (71%), orange crystals, mp 252–253°C.

IR spectrum, ν, cm^{-1}: 1582 (C=N), 394 (Mn←N), 475 (Mn–O). EA spectrum, λ_{max}, cm^{-1}: 27700 ($n \rightarrow \pi*$), 36872, 31401 ($\pi \rightarrow \pi*$). μ_{eff}, B.M.: 5.81. EPR spectrum: *g* 1.983. Found, %: C 32.09, H 2.31, N 16.97, Mn 16.14. $C_9H_8N_4O_3FMnCl$. Calculated, %: C 32.77, H 2.44, N 16.97, Mn 16.65. *M* 327.15 (calculated 329.78).

Aquochloro[2-(5-fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarbothioamide]manganese(II) (2). Yield 1.27 g (78%), light orange crystals, mp 254–255°C. IR spectrum, ν, cm^{-1}: 1592 (C=N), 396 (Mn←N), 315 (Mn–S). EA spectrum, λ_{max}, cm^{-1}: 27855 ($n \rightarrow \pi*$), 37177, 31546 ($\pi \rightarrow \pi*$). μ_{eff}, B.M.: 5.88. EPR spectrum: *g* 2.013. Found, %: C 30.87, H 2.10, N 16.20, S 9.17, Mn 15.67. $C_9H_8N_4SClFO_2Mn$. Calculated, %: C 31.25, H 2.33, N 16.26, S 9.27, Mn 15.88. *M* 344.97 (calculated 345.83).

Aquochloro[2-(5-bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarboxamide]manganese(II) (3). Yield 1.28 g (79%), yellow crystals, mp 250–251°C. IR spectrum, ν, cm^{-1}: 1575 (C=N), 386 (Mn←N), 465 (Mn–O). EA spectrum, λ_{max}, cm^{-1}: 27397 ($n \rightarrow \pi*$), 36634, 31252 ($\pi \rightarrow \pi*$). μ_{eff}, B.M.: 5.77. EPR spectrum: *g* 2.011. Found, %: C 29.19, H 2.04, N 15.23, Mn 15.11. $C_9H_8N_4BrClO_3Mn$. Calculated, %: C 30.13, H 2.24, N 15.68, Mn 15.31. *M* 357.19 (calculated 358.71).

Aquochloro[2-(5-bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarbothioamide]manganese(II) (4). Yield 1.36 g (74%), yellow crystals, mp 250–251°C. IR spectrum, ν, cm^{-1}: 1597 (C=N), 393 (Mn←N), 310 (Mn–S). EA spectrum, λ_{max}, cm^{-1}: 27624 ($n \rightarrow \pi*$), 36756, 31371 ($\pi \rightarrow \pi*$). μ_{eff}, B.M.: 5.91. EPR spectrum: *g* 2.014. Found, %: C 26.22, H 1.77, N 13.46, S 7.54, Mn 13.46. $C_9H_8N_4SClBrO_2Mn$. Calculated, %: C 26.57, H 1.98, N 13.83, S 7.88, Mn 13.50. *M* 405.91 (calculated 406.75).

Bis[2-(5-fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarboxamide]manganese(II) (5). Yield 1.56 g (69%), orange crystals, mp 239–240°C. IR spectrum, ν, cm^{-1}: 1590 (C=N), 398 (Mn←N), 480 (Mn–O). EA spectrum, λ_{max}, cm^{-1}: 27710 ($n \rightarrow \pi*$), 36873, 31403 ($\pi \rightarrow \pi*$). μ_{eff}, B.M.: 5.83. EPR spectrum: *g* 1.995. Found, %: C 42.78, H 2.34, N 22.56, Mn 10.91. $C_{18}H_{12}N_8O_4F_2Mn$. Calculated, %: C 43.43, H 2.42, N 22.60, Mn 11.03. *M* 497.31 (calculated 497.70).

Bis[2-(5-fluoro-2-dihydro-2-oxo-1*H*-indol-3-ylidene)-hydrazinecarbothioamide]manganese(II)] (6). Yield 1.83 g (76%), light orange crystals, mp 241—42°C. IR spectrum, ν, cm^{-1}: 1594 (C=N), 401 (Mn←N), 324 (Mn–S). EA spectrum, λ_{max}, cm^{-1}: 27860 ($n \rightarrow \pi*$), 37176, 31548 ($\pi \rightarrow \pi*$). μ_{eff}, B.M.: 5.95. EPR spectrum: *g* 2.013. Found, %: C 40.13, H 2.01, N 20.66, S 11.93, Mn 10.32. $C_{18}H_{12}N_8S_2F_2O_2Mn$. Calculated, %: C 40.80, H 2.28, N 21.23, S 12.10, Mn 10.36. *M* 528.67 (calculated 529.84).

Bis[2-(5-bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarboxamide]manganese(II) (7). Yield 1.94 g (69%), yellow crystals, mp 258–259°C. IR spectrum, ν, cm^{-1}: 1583 (C=N), 389 (Mn←N), 471 (Mn–O). EA spectrum, λ_{max}, cm^{-1}: 27400 ($n \rightarrow \pi*$), 36639, 31256 ($\pi \rightarrow \pi*$). μ_{eff}, B.M.: 5.82. EPR spectrum: *g* 2.012. Found, %: C 34.54, H 1.44, N 18.05, Mn 8.56. $C_{18}H_{12}N_8Br_2O_4Mn$. Calculated, %: C 34.89, H 1.95, N 18.16, Mn 8.86. *M* 619.36 (calculated 619.51).

Bis[2-(5-bromo-2-dihydro-2-oxo-1*H*-indol-3-ylidene)hydrazinecarbothioamide]manganese(II) (8). Yield 2.16 g (73%), light yellow crystals, mp 272–273°C. IR spectrum, ν, cm^{-1}: 1590 (C=N), 397 (Mn←N), 318 (Mn–S). EA spectrum, λ_{max}, cm^{-1}: 27635 ($n \rightarrow \pi*$), 36759, 31370 ($\pi \rightarrow \pi*$). μ_{eff}, B.M.: 5.93. EPR spectrum: *g* 2.006. Found, %: C 33.10, H 1.57, N 17.12, S 9.79, Mn 8.40. $C_{18}H_{12}N_8S_2Br_2O_2Mn$. Calculated, %: C 33.28, H 1.85, N 17.26, S 9.83, Mn 8.42. *M* 650.53 (calculated 651.83).

Biological activity testing. The antibacterial activity testing of ligands LH and their manganese complexes **1**–**8** against *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) was performed by the Kirby–Bauer disk diffusion method, using the Müeller Hinton agar (HiMedia Labs, Mumbai, India) [45, 46]. The agar medium (17 g) was added into a Petri dish and allowed to solidify. The pH of the agar was maintained between 7.2 and 7.4 at room temperature. The compounds were dissolved in dimethyl sulphoxide (DMSO) in 500 and 1000 ppm concentrations. Whatman no.1 filter paper disks (diameter 6 mm) were soaked in these solutions, dried, and placed on the medium preliminarily seeded with the test organisms in Petri plates at suitable distances. The Petri plates were stored in an incubator at 35°C for 24 h. Streptomycin disc 6 mm in size was used as standard antibiotic (+ve control). The zone of inhibition around each disc containing the test compounds was measured precisely in mm.

The activity indices for the test compounds were calculated by the formula:

$$\text{Activity indices} = \frac{Z_{\text{test}}}{Z_{\text{standard}}} \times 100\%.$$

Here Z_{test} is zone of inhibition of test compounds (diameter in mm) and Z_{standard} is zone of inhibition of standard compounds (diameter in mm).

The antifungal activity testing of ligands LH and their manganese complexes **1**–**8** against *Fusarium semitectum* (ATCC 200360) and *Aspergillus flavus* (ATCC 204304) was performed by the well diffusion method [47]. Each test culture was streaked on to a non-inhibitory agar medium to obtain isolated colonies. After incubation at 35°C overnight, 4 or 5 well-isolated colonies were selected with an inoculum's needle and were transferred to a tube of sterile saline or nonselective broth. The inoculum was prepared using yeasts from a 24-h culture on sabouraud dextrose agar (prepared by dissolving 65 g of sabouraud dextrose in 1000 $cm^3$of distilled water). Sabouraud dextrose agar (20 cm^3 in Petri dishes 8 cm in diameter) was used as the solid medium for preparing wells. The test compounds were introduced in wells (6 mm) as DMSO solutions (*c* 50, 100, and 200 ppm). The Petri plates were wrapped in polythene bags containing a few drops of alcohol, and placed in an incubator at 35°C for 24-48 h. The controls were also run, and three replicates were used in each case. After incubation, the diameter of the zones of complete inhibition (including the diameter of the well) was measured, and recorded in ppm. The linear growth of fungi was obtained by measuring the diameter of the fungal colony after 4 days. The percent inhibition was calculated by the formula:

$$I = \frac{C - T}{C} \times 100\%.$$

Here I is the inhibition (%); C, diameter of the fungal colony in the control plate after 96 h, (mm); and T, diameter of the fungal colony in the test plates after the same period. Itraconazole was used as standard antifungal agent (+ve control). The antibacterial and antifungal activity testing are listed in Tables 2 and 3, respectively.

DNA cleavage activity testing. The primary culture of *E. coli* (ATCC 25922) was inoculated on nutrient broth (peptone 5 g, beef extract 3 g, sodium chloride 5 g, distilled water 1000 cm^3, pH 7.0), autoclaved at 121°C and 15 psi for 15 min. The seeded media was incubated at 37°C for 24h in a shaker at 180 rpm. The secondary culture was obtained by transferring the primary culture to an equal amount (25 mL primary culture and 25 mL fresh medium) of a fresh nutrient broth. After 48-h incubation, DNA was isolated. Fresh bacterial culture, 1 cm^3, was centrifuged at 6000 rpm for 10 min to obtain a pellet. To this pellet, 0.25 cm^3 of cell lysis buffer (0.10 mmol/cm^3 tris pH 8.0, 0.05 mmol/cm^3 EDTA, and 0.05 mmol/cm^3 lysozyme) was added, and centrifuging was continued at 10000 rpm for 10 min. The supernatant was separated and incubated for 1 h at –20°C and, after addition of 0.25 cm^3 of saturated phenol, chloroform, and isoamyl alcohol (25 : 24 : 1 v/v), the mixture was centrifuged. The upper aqueous layer was separated, diluted with a double amount of chilled ethanol and 0.05 cm^3 of sodium acetate (3 mol/L), centrifuged to precipitate DNA. The precipitate was dried, dissolved in TAE buffer (0.10 mmol/cm^3 tris pH 8.0 adjusted with glacial acetic acid, 0.01 mmol/cm^3 EDTA) and stored in cold. Solutions of the test compounds in DMSO (*c* 5μg/cm^3) were added separately to the DNA sample. The DNA-compound mixtures were incubated at 37°C for 2 h [48]. The samples (0.10 cm^3 DNA-compound mixtures+bromophenol blue dye in 1 : 1 ratio) were electrophoresed for 30 min at 50 V on 1% agarose gel using TAE buffer, pH 8.3. After electrophoresis, the gel was removed and stained with 10.0 μg/cm^3 ethidium bromide for 10–15 min, and the bands were visualized and interpreted under a UV transilluminator.

CONCLUSIONS

On the basis of the analytical and spectral evidence we can suggest that the synthesized complexes have a tetrahedral geometry. The biological screening results of the ligands and their metal complexes show that the complexes are more potent antimicrobial agents than the parent ligands due to the chelation. The complexes showed a high DNA cleavage activity. The results of the present research can be useful in the development of possible applications in the analytical, biological, and pharmaceutical fields.

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