



Synthesis, Characterization and Biological Activity of ONO Donor Schiff Base and its Metal Complexes

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A new Schiff base ligand (*E*)-2-((7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)methylene)-*N*-(5-methyl-3-phenyl-1*H*-indol-2-yl)hydrazine carboxamide (**L**) (**3**) was synthesized by the reaction of *N*-(5-methyl-3-phenyl-1*H*-indol-2-yl)hydrazinecarboxamide (**1**) and 7-hydroxy-4-methyl-2-oxo-2*H*-chromene-8-carbaldehyde (**2**). The Cu(II), Co(II), Ni(II) and Zn(II) metal complexes (**4a-d**) were synthesized and its structural elucidation was done by different spectral techniques. The Schiff base (**3**) behaves as ONO donor ligand and forms the complexes of the sort [M(L)(Cl)(H₂O)₂] for Cu(II) (**4a**) and Zn(II) (**4d**) and [M(L)₂] for Co(II) (**4b**) and Ni(II) (**4c**). Compounds (**3**) and (**4a-d**) were tested *in vitro* for antimicrobial action, cytotoxicity property against *Artemia salina* and anti-tuberculosis assay against *Mycobacterium tuberculosis* (ATCC 25177). The metal complexes showed very good biological activity.

Keywords: Coumarin, Indole, Cytotoxicity, Schiff base, Transition metal(II) complexes, Antimicrobial activity, Anti-tuberculosis.

INTRODUCTION

Schiff bases, which are generated from an amino and carbonyl molecule, are a well-studied class of ligands that coordinate to metal ions *via* azomethine nitrogen. The C=N linkage is required for biological activity in azomethine derivatives, and various azomethine derivatives have been shown to have exceptional antibacterial, antifungal, anticancer, and antimalarial properties [1]. The Schiff base ligands have been extensively investigated in coordination chemistry, owing to their ease of availability, synthesis and electrical characteristics [2]. Schiff base-coordination chemistry has recently gained a lot of interest because of its important functions in electroplating, metal refining, analytical chemistry, organic synthesis, metallurgy and photography [3]. The azomethine nitrogen of Schiff bases provides a binding site for metal ions to be coupled to various biomolecules such as proteins and amino acids for anti-germ actions in biological systems [4].

Indole analogues are an important class of organic heterocyclics that have been used to treat a variety of diseases through

out history. They are also one of the most appealing frameworks with a diverse spectrum of biological activities [5] and one of the most active compounds in heterocycles which acquiring enormous importance within the field of medicinal chemistry. Indole and its derivatives also are present in many natural products like plants, fungi and marine organisms, *etc.* [6]. Furthermore, the biological profile of a mixture of two or more heterocyclic moieties is many times that of the parent indole nucleus [7], thus they must be brought to the attention of chemists to encourage them to compete in the synthesis of new biologically active molecules. Because of the presence of an electron rich pyrrole component, indole and its derivatives are electron-rich aromatic compounds with distinct characteristics. It is extensively distributed in natural systems as a key component of biomolecules and natural products [8].

Coumarin and its derivatives are of great importance due to their vital role in synthetic and natural organic chemistry. The compounds which contain coumarin moiety display good bioactivities like hypnotic, molluscicides, insecticidal, anthelmintic, anticoagulant agents and fluorescent brighteners [9].

As a result, coumarins with a Schiff base are expected to have increased biological activity. In this work, a new Schiff base ligand as well as its transition metal(II) complexes comprising the indole and coumarin moiety are synthesized, characterized, and also evaluated their biological effects.

EXPERIMENTAL

The precursors *N*-(5-methyl-3-phenyl-1*H*-indol-2-yl)-hydrazinecarboxamide (**1**) and 7-hydroxy-4-methyl-2-oxo-2*H*-chromene-8-carbaldehyde (**2**) were synthesized by the literature methods [10,11]. The metal and chlorides content was estimated gravimetrically [12]. All the chemicals and solvents utilized in the synthesis were chemically pure and used as supplied.

The elemental analyses were administered employing a CHNS analyzer (Vario EL III). The conductivity was measured in 10^{-3} M DMF at room temperature using Conductivity Bridge Elico-CM 180. Sherwood MSB mark1 Gouy balance is used to measure the magnetic susceptibility. KBr discs were used in IR spectrum analysis using a Perkin Elmer Spectrum RX-I FTIR spectrophotometer. The Elico-SL 164 double beam spectrophotometer was used to obtain UV-Visible spectra. The proton NMR data was recorded using Bruker Advance DPX spectrometer at 400 MHz and mass spectra were recorded on instrument equipped with an electron ionization supply.

Synthesis of Schiff base ligand (E)-2-((7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)methylene)-*N*-(5-methyl-3-phenyl-1*H*-indol-2-yl)hydrazine carboxamide (L) (3**):** Ligand (**L**) (**3**) was synthesized by addition of equivalent amount (0.001 mol) of *N*-(5-methyl-3-phenyl-1*H*-indol-2-yl)hydrazine carboxamide (**1**) and 7-hydroxy-4-methyl-2-oxo-2*H*-chromene-8-carbaldehyde (**2**) and refluxed with stirring for about 5-6 h by adding 2-3 drops of acetic acid. The obtained solid compound was filtered and washed using hot ethanol followed by recrystallization using 1,4-dioxane and dried (**Scheme-I**).

Synthesis of Cu(II), Co(II), Ni(II) and Zn(II) metal complexes (4a-d): Equimolar quantities of respective metal salts (0.002 mol in 20 mL of ethanol) were added to ligand (**3**) (0.002 mol in 25 mL of ethanol) to synthesize the metal(II) complexes. The reaction mixture was refluxed for 2 h while stirring and the solid complexes were filtered, washed with hot ethanol and dried over calcium chloride.

Antimicrobial activity: Antibacterial and antifungal activity of newly synthesized compounds **3** and (**4a-d**) were determined using the Muller-Hinton and potato dextrose agar (PDA) by disc and well diffusion techniques, respectively. The assay was performed in several concentrations (100, 50, 25,

12.5 6.25 and 3.125 $\mu\text{g/mL}$) in DMSO solvent. Antibacterial activity was tested using the MIC method [13] against, *Bacillus subtilis* (MTCC 736), *Staphylococcus aureus* (MTCC 3160), *Salmonella typhi* (MTCC 98), *Escherichia coli* (MTCC 46), and antifungal activity against, *Cladosporium oxysporum* (MTCC 1777), *Candida albican* (MTCC 227), *Aspergillus niger* (MTCC 1881) and *Aspergillus flavus* (MTCC 1883). The findings were compared to those of the conventional antibiotics drugs gentamycin and fluconazole.

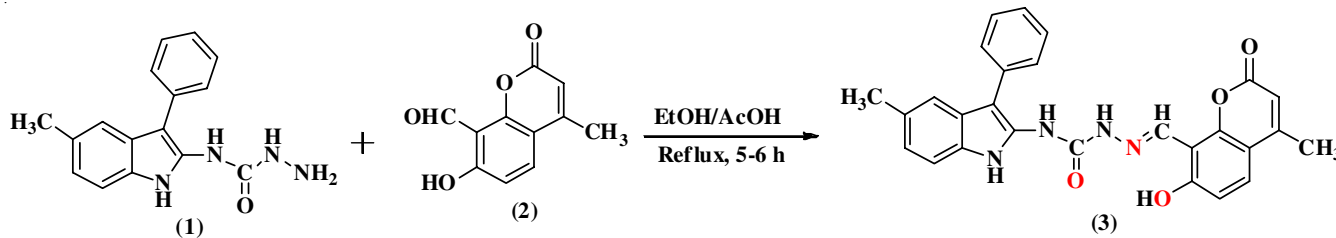
Anti-tuberculosis (Anti-TB) activity: By adopting the literature approach [14] of dispersed culture technique using Kirchner's media with Tween-80, an *in vitro* anti-TB experiment was performed against the human virulent strain *Mycobacterium tuberculosis* (ATCC 25177). The 0.5 mL sterile normal bovine serum, inactivated by heating at 56 °C for 30 min, was added to sterile Kirchner disperse medium (4.5 mL) scattered in the tube (150 × 20 mm). Compounds **3** and **4a-d** were dissolved in DMF and added as a solution to provide final concentration of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 $\mu\text{g/mL}$, with inoculums consisting of 0.1 mL of a standard suspension of *M. tuberculosis* (ATCC 25177) with 10^6 bacilli per milliliter. After an eight-day incubation period at 37 °C, the tubes were checked for the presence or absence of test organism growth. The endpoint was set at the lowest concentration that demonstrated no observable growth. The MIC for compounds **3** and **4a-d** was determined, and the findings were compared to a standard drug control tube containing ciprofloxacin and a tube containing no drug, *i.e.* only DMF.

in-vitro cytotoxicity: Compounds **3** and **4a-d** were tested for their cytotoxicity property (brine shrimp bioassay) by following the reported method [15-18].

RESULTS AND DISCUSSION

All of the synthesized metal(II) complexes (**4a-d**) are non-hygroscopic coloured solids, which are stable in air and only sparingly soluble in typical organic solvents, but totally soluble in DMF and DMSO. Complexes **4a** and **4d** have 1:1 stoichiometry of the sort $[\text{M}(\text{L})(\text{Cl})(\text{H}_2\text{O})_2]$, while the complexes **4b** and **4c** have 1:2 stoichiometry of the sort $[\text{ML}_2]$, where L stands for deprotonated ligand, according to the basic analysis. The molar conductance values are far too low to account for non-electrolyte dissociation in DMF [19,20]. Table-1 shows the physico-chemical and analytical data for ligand (**3**) and its metal(II) complexes (**4a-d**).

IR spectral studies: Schiff base ligand **3** has a broad IR band due to the phenolic -OH group at 3420 cm^{-1} , and three bands at 3232, 3048 and 3020 cm^{-1} due to the indole NH,



Scheme-I: Preparation of Schiff base Ligand (L) (**3**)

TABLE-1
PHYSICO-CHEMICAL AND ANALYTICAL DATA OF SCHIFF BASE
LIGAND (L) (3) AND ITS Cu(II), Co(II), Ni(II) AND Zn(II) COMPLEXES 4(a-d)

Ligand/ complexes	m.w. (g)	m.p. (°C)	Colour (Yield, %)	Elemental analysis (%): Calcd. (found)					λ_m (Ω^{-1} cm ² mol ⁻¹)	μ_{eff} (BM)
				C	H	N	M	Cl		
C ₂₇ H ₂₂ N ₄ O ₄ (L) (3)	466.00	274	Yellow (80)	69.52 (69.60)	4.72 (4.64)	12.01 (12.09)	–	–	–	
[Cu(L)(Cl)(H ₂ O) ₂] (4a)	599.54	> 300	Green (70)	54.04 (54.09)	4.16 (4.10)	9.34 (9.26)	10.59 (10.65)	5.83 (5.88)	30	1.80
[Co(L) ₂] (4b)	988.93	> 300	Brown (64)	65.52 (65.61)	4.24 (4.12)	11.32 (11.41)	5.95 (5.84)	–	17	5.07
[Ni(L) ₂] (4c)	988.69	> 300	Brown (67)	65.54 (65.61)	4.24 (4.16)	11.32 (11.42)	5.93 (5.80)	–	18	2.91
[Zn(L)(Cl)(H ₂ O) ₂] (4d)	601.40	> 300	Orange (73)	53.87 (53.95)	4.15 (4.05)	9.31 (9.38)	10.87 (10.76)	5.81 (5.89)	28	Diamag.

amide NH, and NH linked to the indole moiety, respectively. The $\nu(\text{C}=\text{O})$ lactone carbonyl, $\nu(\text{C}=\text{O})$ amide carbonyl, $\nu(\text{C}=\text{N})$ azomethine function, and phenolic C-O displayed the high-intensity bands at 1787, 1692, 1633, and 1270 cm⁻¹, respectively.

Weak bands at 3246-3220, 3061-3046 and 3025-3016 cm⁻¹ in the IR spectra of metal(II) complexes (4a-d) were attributable to indole NH, amide NH and NH linked to the indole moiety, respectively, which appeared at about an equivalent region as in case of Schiff base ligand 3, showing their non-involvement in the coordination [21].

Furthermore, the absence of a band attributable to phenolic OH at 3420 cm⁻¹ of Schiff base ligand 3 in its metal(II) complexes (4a-d) suggests that the metal ion and the phenolic oxygen atom of the coumarin moiety formed a coordination link by deprotonation [22]. This is typically corroborated by a sharp increase in the frequency of $\nu(\text{C}-\text{O})$ about 20-55 cm⁻¹, which appeared in the region 1325-1290 cm⁻¹ in the metal(II) complexes (4a-d), indicating the involvement of the phenolic oxygen atom in coordination.

The most noticeable alteration is the shift of $\nu(\text{C}=\text{O})$ frequency to the lower side about 26-70 cm⁻¹, which appeared in the region 1666-1622 cm⁻¹ in the metal(II) complexes (4a-d), confirming the intrinsic involvement of the oxygen atom of $\nu(\text{C}=\text{O})$ in coordination without enolization. In addition, in metal(II) complexes (4a-d), the shift of $\nu(\text{C}=\text{N})$ frequency to the lower side of 29-86 cm⁻¹ and appearance in the region 1604-1547 cm⁻¹ indicates the involvement of nitrogen atom in complexation [23]. The bandwidth at 3364 cm⁻¹ and 3409 cm⁻¹ in the copper (4a) and zinc (4d) metal complexes is owing to coordinated water molecules. Table-2 shows the IR spectra frequencies of the Schiff base ligand (3) and its metal(II) complexes (4a-d), along with their assignments, to demonstrate the essential functional groups and binding modes.

¹H NMR studies: The ¹H NMR spectrum of compound 3 revealed four singlets at 12.5, 11.8, 11.6, and 9.1 ppm, which are attributed to protons of phenolic OH, amide NH, NH linked to indole moiety, and indole NH, respectively, while a singlet proton signal at 8.1 ppm is attributed to azomethine proton (HC=N). The aromatic protons reverberated as a multiplet in the 7.3-7.9 ppm range, and two signals at 2.4 and 2.6 ppm are owing to three protons of the coumarin methyl and indole moieties, respectively.

In diamagnetic 4d complex, the protons of amide NH, NH linked to indole moiety, and indole NH are attributed to singlet proton signals at 12.1, 11.6, and 10.5 ppm, respectively, while singlet proton signal at 8.3 ppm is ascribed to azomethine proton (HC=N). The aromatic protons reverberated as a multiplet in the 7.1-7.5 ppm range, while two signals at 2.4 and 2.5 ppm are owing to three protons from the coumarin and indole moiety's methyl groups, respectively.

The signal due to phenolic OH group of Schiff base ligand (3), which was observed at 12.5 ppm, has completely disappeared in 4d complex, showing that phenolic oxygen atoms are involved and connecting with metal ions *via* deprotonation. Four protons of two coordinated water molecules correlate to the appearance of a new signal at 3.8 ppm.

Mass spectral studies: The molecular ion peaks in the mass spectra of the substances corresponded to their molecular weights. The M+1 peak was observed at 466 (60%) in the ESI mass spectrum of Schiff base ligand (3). The molecular ion peak was detected at M+ 599, 601 (9%, 3%) and 988 (40%) in the metal(II) complexes 4a and 4b respectively, along with other fragment peaks.

Electronic spectral studies: In the range of 15200-17926 cm⁻¹, the copper(II) complex (4a) showed a single broad asymmetric band of low intensity. The width of the band denotes

TABLE-2
IR SPECTRAL DATA OF FREE LIGAND (L) (3) AND ITS METAL COMPLEXES 4(a-d)

Ligand/ complexes	$\nu(\text{OH})$	$\nu(\text{H}_2\text{O})$	$\nu(\text{NH})$	$\nu(\text{NH})$	$\nu(\text{NH})$	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}-\text{O})$
3	3420	–	3232	3048	3020	1787	1692	1633	1270
4a	–	3364	3236	3049	3022	1773	1662	1598	1297
4b	–	–	3220	3046	3025	1756	1666	1604	1298
4c	–	–	3240	3048	3021	1792	1622	1547	1325
4d	–	3409	3246	3061	3016	1753	1657	1604	1290

the three transitions ${}^2B_{1g} \rightarrow {}^2A_{1g}$ (ν_1), ${}^2B_{1g} \rightarrow {}^2B_{2g}$ (ν_2) and ${}^2B_{1g} \rightarrow {}^2E_g$ (ν_3), which are energetically similar and only provide a wide absorption band, and so the width of the band may be attributed to Jahn Teller distortion. All of these findings suggested that the Cu(II) ion has a deformed octahedral shape [24,25]. The cobalt(II) complex's (**4b**) electronic spectra revealed two absorption bands at 16716 cm^{-1} and 19776 cm^{-1} . The ${}^4T_{1g}$ (F) \rightarrow ${}^4A_{2g}$ (F) (ν_2) and ${}^4T_{1g}$ (F) \rightarrow ${}^4T_{2g}$ (P) (ν_3) transitions are assigned to these bands, which are in good agreement with the reported values [26]. Due to the limited range of the instrument utilized, the lowest band, ν_1 could not be seen, but it could be approximated using the band fitting approach proposed by Underhill & Billing [26]. In Table-3, the computed value for ν_1 is shown. The octahedral geometry of the Co(II) complex is suggested by the obtained transition values of ν_1 , ν_2 and ν_3 . Within the region 14846 cm^{-1} and 25230 cm^{-1} , the complex **4c** showed two absorption bands, which were ascribed to the ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F) (ν_2) and ${}^3A_{2g}$ (F) \rightarrow ${}^3T_{1g}$ (P) (ν_3) transitions, respectively. A band fitting approach was used to calculate the transition value of band ν_1 [27]. Table-3 shows the electronic spectrum data and ligand field characteristics of Cu(II), Co(II), and Ni(II) complexes (**4a-c**).

The calculated values of ligand field parameters such as nephelauxetic parameter (β), ligand field splitting energy (10 Dq), Racah inter electronic repulsion parameter (B') and ligand field stabilization energy (LFSE) [28] further supported the proposed octahedral geometry of metal(II) complexes **4a**, **4b** and **4c**. Because of orbital overlap and delocalization of *d*-orbitals, the computed B' values for metal(II) complexes **4b** and **4c** are lower than the free ion values. The values are essential in assessing the covalency of the metal-ligand link and the fact that they are smaller than unity indicates that the metal-ligand bonds have a significant amount of covalency. Nickel(II) complex (**4c**) had a lower β value than the cobalt(II) complex (**4b**), indicating that the M-L bond has a higher covalency.

Magnetic susceptibility studies: For metal(II) complexes (**4a-c**), the paramagnetic behaviour was confirmed by magnetic susceptibility measurements. The observed value for copper(II) complex (**4a**) is 1.80 B.M., which is slightly above the spin-only value due to a unpaired electron 1.73 B.M., which suggests octahedral geometry [29]. As a result, there is no spin interaction with distorted octahedral geometry in this copper(II) complex (**4a**). The ground state of the octahedral cobalt complex (**4b**) is ${}^4T_{1g}$. The magnetic moment values for the various **4b** complexes are within the range 4.12-4.70 B.M. for tetrahedral geometry and 4.70-5.20 B.M. for octahedral geometry, respectively [30]. The magnetic moment value for cobalt(II) complex (**4b**) is 5.07 B.M. in the current measurements, implying the octahedral shape. The measured magnetism for nickel(II) complex (**4c**) is 2.91 BM, which is substantially within the anticipated range of 2.83-3.50 B.M., implying that its octahedral environment is consistent [31].

Antimicrobial activity: Antimicrobial screening was performed with various bacteria and the newly synthesized metal(II) complexes (**4a-d**) out performed the free Schiff base ligand. Schiff base ligand. The activity was increased with the complexation. Chelation theory [32] was used to explain the increase in activity of the complexes above the free Schiff base ligand. The increase in activity could also be explained by the fact that most ligands have azomethine (C=N) linkages. Furthermore, the metal ions' positive charge is shared in part with the heteroatoms (O and N), and there may be π -electron delocalization throughout the chelating system [33]. As a result, metal chelates become more lipophilic, they are more likely to pass through the lipid layer of bacterial membranes and block metal-binding sites in microorganism enzymes. Metal complexes are generally more active than ligands since metal complexes can act as a vehicle for ligand activation due to the main cytotoxic species [34]. Table-4 shows the minimum inhibitory concentrations (MIC g mL^{-1}) for the antibacterial

TABLE-3
ELECTRONIC AND LFSE PARAMETERS OF COMPLEXES **4(a-c)**

Complexes	Transitions (cm^{-1})			Dq (cm^{-1})	B' (cm^{-1})	β	$\beta\%$	ν_2/ν_1	LFSE (kcal)
	ν_1^*	ν_2	ν_3						
4a		15200-17926		–	–	–	–	–	28.39
4b	7806	16716	19776	891	872	0.898	10.19	2.14	15.27
4c	9060	14846	25230	906	858	0.825	17.50	1.63	31.06

TABLE-4
MIC VALUES FOR THE *in vitro* ANTIMICROBIAL, ANTI-TB AND
CYTOTOXICITY RESULTS OF LIGAND (**3**) AND **4(a-d)** COMPLEXES

Compounds	Antibacterial MIC (mg mL^{-1})				Antifungal MIC (mg mL^{-1})				Anti-TB MIC (mg mL^{-1})	Cytotoxicity
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>C. oxysporum</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>M. tuberculosis</i>	Brine shrimp
3	100.00	50.00	50.00	100.00	100.00	50.00	25.00	50.00	12.50	2.121×10^{-4}
4a	25.00	25.00	12.50	25.00	50.00	25.00	25.00	25.00	3.125	1.260×10^{-4}
4b	50.00	25.00	12.50	12.50	50.00	25.00	25.00	25.00	6.25	2.102×10^{-4}
4c	50.00	25.00	25.00	25.00	25.00	50.00	12.50	12.50	3.125	1.099×10^{-4}
4d	12.50	25.00	25.00	25.00	25.00	25.00	12.50	12.50	3.125	1.118×10^{-4}
Gentamicin	12.50	12.50	12.50	12.50	–	–	–	–	–	–
Fluconazole	–	–	–	–	12.50	12.50	12.50	12.50	–	–
Ciprofloxacin	–	–	–	–	–	–	–	–	3.125	–

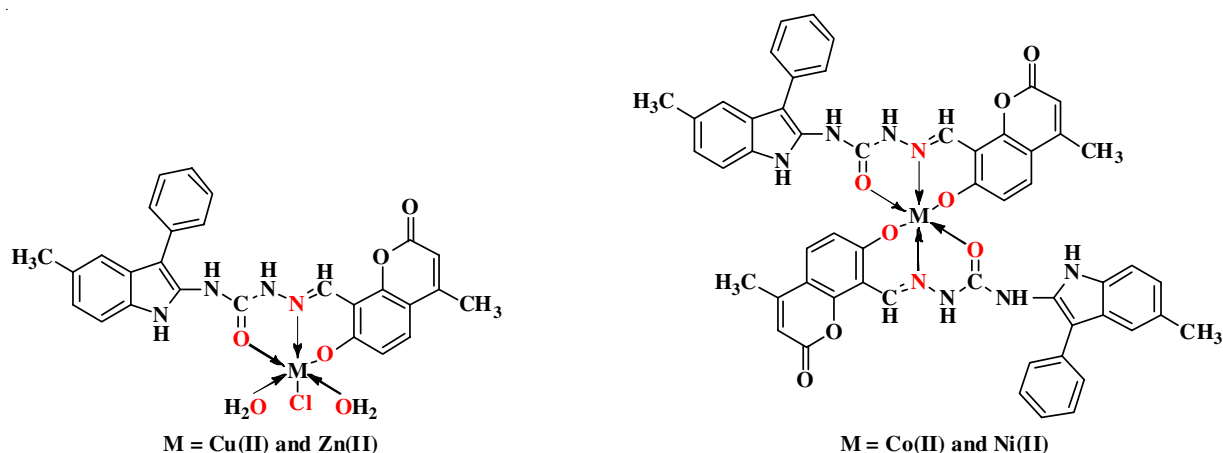


Fig. 1. Proposed structures of the metal complexes 4(a-d)

and antifungal results of Schiff base ligand and its metal(II) complexes against the various bacterial and fungal strains.

Anti-TB studies: The anti-tubercular activity of Schiff base ligand and its metal(II) complexes (**4a-d**) is quite promising as shown in Table-4. The metal(II) complexes **4a**, **4c** and **4d** were shown to be the most active among the screened compounds, with a MIC of 3.125 µg/mL and activity comparable to that of the standard medication ciprofloxacin against *M. tuberculosis* bacteria.

In vitro cytotoxicity studies: Table-4 shows that all the newly metal(II) complexes (**4a-d**) have good activity when compared to the free Schiff base ligand (**3**). The cobalt(II) and zinc(II) complexes (**4c** and **4d**) had substantial cytotoxic action against *Artemia salina*, with LD₅₀ values of 1.099 × 10⁻⁴ and 1.118 × 10⁻⁴ M/mL, respectively.

Conclusion

A newly synthesized Schiff base ligand, (*E*)-2-((7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)methylene)-*N*-(5-methyl-3-phenyl-1*H*-indol-2-yl)hydrazinecarboxamide (**3**) functions as a tridentate ONO donor. The octahedral geometry for all its metal(II) complexes has been postulated using several physico-chemical and spectroscopic approaches (Fig. 1). After complexation, the newly synthesized metal(II) complexes (**4a-d**) demonstrated increased antibacterial activity. The cytotoxicity of the nickel(II) (**4c**) and zinc(II) (**4d**) complexes suggests the presence of powerful cytotoxic agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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