



Synthesis, Spectroscopic Characterization and Biological Studies of Some Metal(II) Complexes of Nicotinic Hydrazide Moiety

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Received: 29 January 2025;

Accepted: 3 March 2025;

Published online: 29 March 2025;

AJC-21945

In this study, a new ligand N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)nicotinohydrazide was synthesized and characterized. Then, the ligand was reacted with Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) metal ions affording its metal(II) complexes. Through elemental analysis, molar conductance, magnetic data, UV-visible, FT-IR, ¹H NMR and mass spectra, the structural characterization of all the metal(II) complexes were accomplished. The FT-IR data demonstrated that in the metal(II) complexes, the ligand chelated to metal ion in a tridentate manner. The UV-visible spectral and magnetic data showed that the complexes exhibit octahedral geometry. Additionally, ligand molecular docking studies were conducted. The antimicrobial study demonstrated that complex formation increased the free ligand activity. The Cd(II) complex is an efficient antifungal agent, whereas the Zn(II) and Cd(II) complexes may be regarded as promising antibacterial agents. Moreover, Cd(II) complex also demonstrated strong anti-TB properties against *M. tuberculosis*. The antioxidant activity results showed that the except Cd(II) complex, other metal(II) complexes were found to be more active than the parent ligand.

Keywords: Nicotinic hydrazide, Antimicrobial activity, Antioxidant activity, Transition metal(II) complexes.

INTRODUCTION

The chemistry of Schiff bases has been intensively investigated in recent years owing to their pharmaceutical activity, the tendency towards the formation of complexes with almost all the transition metals and their diverse applications [1-3]. Heterocycles made of nitrogen are more common than those made of oxygen or sulphur. According to US FDA-approved pharmaceuticals database, 59% of small molecule drugs contain a heterocyclic moiety with a nitrogen atom in it [4]. Six-membered heterocyclic compounds with nitrogen have become particularly important in the realm of drug research [5-8]. Hydrazones are a unique class of organic compounds since they include an azomethine group. In last 20 years, they have frequently been utilized as ligands in coordination chemistry [9,10]. In addition, pyridine derivatives of hydrazones and their chelates of metal have remarkable pharmaceutical growing importance and a several kind of biological processes, including analgesic [4,11], anti-HIV [12], anti-tubercular [13], antimicrobial [14-17], anti-inflammatory [18], antifungal [19], anticonvulsant [20], anti-plasmodial [21] and anticancer [22-27] activities.

The issue of multidrug-resistant bacteria has grown significantly over the past few decades [28]. As a result, a biomedical issue is the synthesis of novel materials used for the efficient and side-effect-free therapy of microbial illnesses. Derivatives of nicotinic acid are substances that occur naturally. Among the most well-known and effective medications for treating human tuberculosis is nicotinic acid hydrazides, which were initially used in tuberculosis therapy in 1950. According to estimates, more than one-third of the world's population is infected with tuberculosis [29], which results in two to three million annual deaths [2,30].

As a ligand for the synthesis of coordination compounds, coumarin-containing molecules have significant applications [1]. We are particularly interested for the transition metal(II) complexes of coumarin derivatives due to the fact that these compounds have shown to have promising biological and medical applications [31,32]. Moreover, they exhibit exceptional thermal stability and optical properties [2,33]. Recent advancements have been made in the research on the cytotoxic effects of coumarin containing nicotinic acid hydrazide moieties and their coordination complexes [4]. In some instances, the resul-

ting metal complexes exhibit higher biological activity than its ligand.

Considering the previously addressed significant features, in this work, a novel Schiff base ligand **L** synthesized from (3-pyridyl-carbonyl)hydrazine and 3-acetyl-2*H*-1-benzopyran-2-one, is utilized to prepare the few transition metal(II) complexes *viz.* Co(II), Ni(II), Cu(II), Zn(II) and Cd(II), characterized and evaluated for their diverse biological activities. The Schiff base ligand was also evaluated for docking study with lanosterol 14 α -demethylase (4WMZ) [34].

EXPERIMENTAL

All the chemicals and solvents were acquired *via* HiMedia and Sigma-Aldrich. The solvents were purified and dried using standard methods [35]. Elemental UNICUBE was employed to measure the percentages of C, H and N. The melting points were measured without correction using open capillaries and electrothermal equipment. To electronic spectral measurements in DMSO were performed using the Thermo-Scientific Genesys 150 UV-visible spectrophotometer. The Perkin-Elmer FT-IR spectrometer was used to capture the FTIR spectra. ^1H NMR spectra in DMSO- d_6 were captured using a Bruker Advance Neo 500 MHz. An American Model Xevo G2-XS QT was employed to record the mass spectra. At room temperature, the magnetic susceptibilities were measured on a Gouy's balance with a calibrant of $\text{Hg}[\text{Co}(\text{NCS})_4]$. The ELICO-CM-82T conductivity bridge in the DMSO solution was used to record the molar conductance data.

Synthesis of novel Schiff base, N' -(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)nicotinohydrazide (L**):** To a hot ethanolic solution containing 3-pyridylcarbonylhydrazine (**1**) (0.001 mol) and 3-acetyl-2*H*-1-benzopyran-2-one (**2**) (0.001 mol), added two-three drops of glacial acetic acid with vigorous stirring and then refluxed for 3-4 h. Following the completion of reaction, the mixture was stand to cool to normal temperature. The final solid was then collected, washed with cold alcohol, dried and recrystallized (**Scheme-I**).

Synthesis of metal(II) complex: The metal(II) acetates of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) were mixed in 1:2 molar ratio with Schiff base ligand in an ethanolic solution and then refluxed in a water bath for 1 to 2 h. A colourful product emerged after allowing the above reaction mixture to stand and cool. The complexes underwent filtering, a hot ethanol wash and desiccator drying over anhydrous CaCl_2 . In an electric oven, they underwent more drying process.

DPPH radical scavenging activity (RSA): The DPPH free radical scavenging activity of Schiff base and its metal(II)

complexes were measured spectrophotometry at 517 nm. In this work, ascorbic acid (5-35 μM) was used as standard. After adding DPPH (0.2 mM in ethanol), the solution was incubated at room temperature for 30 min in dark. Eqn. 1 was used to calculate the percent inhibition of the DPPH radical scavenger from the absorbance of the solutions and control (DPPH) at 517 nm.

$$\text{Antioxidant activity (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where A_c = absorbance of control and A_s = absorbance of sample.

ABTS $^{+}$ radical scavenging activity (RSA): ABTS $^{+}$ radical scavenging activity method was employed to assess the inhibition of free radicals by ligands and metal(II) complexes [36]. A mixture of ABTS solution (7 mM) and 2.45 mM potassium persulfate was prepared and allowed to incubate at room temperature for 16-18 h in dark. Following the designated time frame, the absorbance of the ABTS radical cation (ABTS $^{+}$) solution was calibrated at 734 nm using distilled water. The ABTS solution (180 μL) was mixed with 20 μL of Schiff base ligand and metal(II) complexes in DMSO, followed by 30 min incubation in dark, after which measurements were taken using a microplate reader [37]. All the experiments were conducted using three replicates. Gallic acid (GA) and butylated hydroxy anisole (BHA) were used as standards. The activity of ABTS $^{+}$ scavenging was determined using eqn. 2:

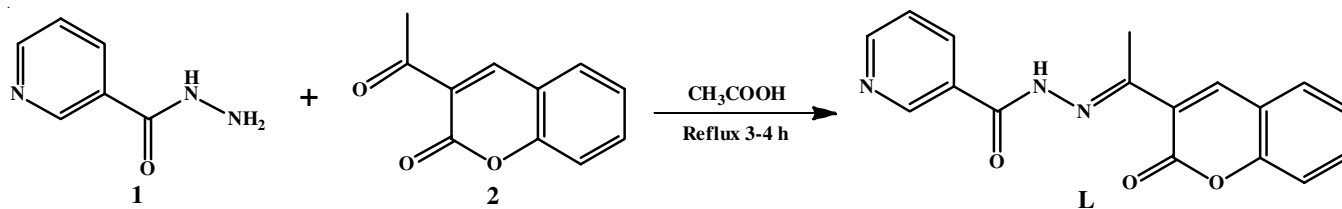
$$\text{Scavenging effect of ABTS (\%)} = \frac{AB - AA}{AB} \times 100$$

where AB represents the absorbance of DMSO and ABTS $^{+}$ mixture, AA refers to the absorbance of sample and ABTS $^{+}$ solution.

Molecular docking study: The molecular docking is an effective computer-aided drug design method for predicting the drug-biomolecule binding in proteins and *ct*-DNA. This study used the molecular docking to study the groove binding characteristics and discover how drugs bind in the active site. To verify the findings, molecular modeling was done. The Schiff base ligand and 14 α -demethylase (4WMZ) enzyme were prepared in Biovia discovery studio by adding hydrogen to the polar atoms and the metal ions' valency was optimized.

RESULTS AND DISCUSSION

The results of elemental analysis data in Table-1, all the confirm that metal(II) complex is in the metal:ligand (1:2) stoichiometric ratio. The synthesized metal(II) complexes showed molar conducting values ranging from 2.52 to 6.36 $\text{S cm}^2 \text{mol}^{-1}$, which suggests that they are non-ionic in nature.



Scheme-I: Synthesis of N' -(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)nicotinohydrazide (**L**)

TABLE-1
ELEMENTAL ANALYSIS AND PHYSICAL CHARACTERISTICS OF SCHIFF BASE LIGAND AND THEIR METAL COMPLEXES

Compd.	Colour	m.p. (°C)	Yield (%)	Formula	Elemental analysis (%): Found (calcd.)				Conductance (S cm ² mol ⁻¹)	μ_{eff} (BM)
					C	H	N	M		
L	Light yellow	182-184	72	C ₁₇ H ₁₃ N ₃ O ₃	66.12 (66.44)	4.19 (4.26)	13.58 (13.67)	–	–	–
CoL	Coffee	282	62	C ₃₄ H ₂₄ N ₆ O ₆ Co	60.78 (60.81)	3.56 (3.60)	12.47 (12.51)	8.74 (8.78)	6.36	4.92
NiL	Brown	301	65	C ₃₄ H ₂₄ N ₆ O ₆ Ni	60.79 (60.83)	3.57 (3.60)	12.48 (12.52)	8.67 (8.74)	5.67	2.93
CuL	Green	251	71	C ₃₄ H ₂₄ N ₆ O ₆ Cu	60.32 (60.40)	3.53 (3.58)	12.36 (12.43)	9.35 (9.40)	2.52	1.73
ZnL	Yellow	214	69	C ₃₄ H ₂₄ N ₆ O ₆ Zn	55.97 (56.03)	3.97 (4.00)	9.72 (9.80)	15.16 (15.25)	3.18	Dia.
CdL	Yellow	294	67	C ₃₄ H ₂₄ N ₆ O ₆ Cd	50.35 (50.49)	3.52 (3.60)	8.71 (8.83)	23.58 (23.63)	3.56	Dia.

FT-IR spectra: Table-2 listed the key IR characteristic frequencies of the Schiff base ligand and its metal(II) complexes. The stretching vibration of the -NH group is responsible for the medium intensity weak band at 3253 cm⁻¹ that was visible in the ligand. A new absorption band at the frequency of 1665-1653 cm⁻¹ and the band disappears corresponding to the NH group upon complexation suggested the coordination of a new C=N group. The carbonyl group of pyridinyl ring, which is in charge to the high strong band at 1659 cm⁻¹ in Schiff base ligand, disappears in the metal(II) complexes and a new band appears at 1744-1717 cm⁻¹, confirming the change of C=O to C–O. The ligand strong bands at 1726 cm⁻¹ and 1643 cm⁻¹ are appeared by the carbonyl group of lactone and the azomethine, respectively. Furthermore, the oxygen atom in lactone carbonyl and the nitrogen atom in azomethine groups interact with metal ions as observed in the shifting of these bands to lower frequencies of 40-12 cm⁻¹ and 5-44 cm⁻¹ and their emergence in 1714-1686 cm⁻¹ and 1638-1599 cm⁻¹ after complexation [38].

Electronic spectra and magnetic susceptibility of metal(II) complexes: The electronic spectra of Co(II) complex show three bands at 10036, 16233 and 20533 cm⁻¹, which are due to the transitions $^4T_{1g} \rightarrow ^4T_{2g}(\text{F})$ (ν_1), $^4T_{1g} \rightarrow ^4A_{2g}(\text{F})$ (ν_2) and $^4T_{1g} \rightarrow ^4T_{1g}(\text{P})$ (ν_3), respectively (Fig. 1a) [39]. In Co(II) complex, we observed a magnetic moment of 4.92 B.M. at ambient temperature, whereas the typical complexes have a magnetic moment of 4.8-5.2 B.M. [36]. The Ni(II) complex is octahedral, as evidenced by its magnetic moment of 2.93 B.M., which ranges between 2.9 and 3.3 B.M. when measured at room temperature [36]. The Ni(II) complex electronic spectrum contains peaks at 7761, 12738 and 21786 cm⁻¹ (Fig. 1b). Each of these bands may be responsible for the transitions $^3A_{2g}(\text{F}) \rightarrow ^3T_{2g}(\text{F})$ (ν_1),

$^3A_{2g}(\text{F}) \rightarrow ^3T_{1g}(\text{F})$ (ν_2) and $^3A_{2g}(\text{F}) \rightarrow ^3T_{1g}(\text{P})$ (ν_3). It suggests an octahedral geometry for the Ni(II) complex [39,40]. The Cu(II) complex magnetic moment of 1.73 B.M., which falls within 1.74 to 1.85 range indicates that it has an octahedral structure [41]. The electronic spectrum produced a wide and low-intensity shoulder band. Tetragonal distortion causes the octahedral Cu(II) ion (d^9) to break into the 2E_g and $^2T_{2g}$ forms [42]. The spectra may not resolve the three transitions $^2B_{1g} \rightarrow ^2B_{2g}$, $^2B_{1g} \rightarrow ^2E_g$ and $^2B_{1g} \rightarrow ^2A_{1g}$ because of the distortion (Fig. 1c). These three transitions were identified as bands of charge transfer between ligand and metal and are located inside a single wide band ranges from 17361-13513 cm⁻¹. In the Cu(II) complex, the deformed octahedral shape is preferred [43].

The values of the electronic parameters, including the covalency factor (B), ligand field splitting energy (10Dq), ligand field stabilization energy (LFSE) and Racah interelectronic repulsion parameter (β') [44,45] were computed using the band-fitting equation [46]. For the complexes, the β' values were not as high as the unbound ions, indicating orbital overlaps and d -orbital delocalization. The complexes covalent factor (β), which is equal to β/β' , was less than unity, implies that metal-ligand linkages are significantly covalent. Furthermore, the values of β were used to compute the covalence factor ($b^{1/2}$), Sinha parameter ($\delta\%$) or metal-ligand covalency percent and the covalency angular overlap parameter (η) using the formulas reported elsewhere [45].

The Co(II) and Ni(II) complexes electronic spectrum analyses show favourable values for $(1-\beta)$, $b^{1/2}$, $\delta\%$ and η , indicating that the metal–ligand interaction in complexes was covalent. According to Table-3, the values of the angular overlap parameter (η) and the bonding parameter ($\beta^{1/2}$) turned out to be

TABLE-2
FT-IR SPECTRA BANDS OF THE LIGAND AND ITS COMPLEXES

Ligand/ complex	m.f.	m.w.	FT-IR (cm ⁻¹)							
			N-H	C=N (coumarin)	C=N (pyridinyl)	C=O (lactone)	C=O (amide)	C-O	M-O	M-N
L	C ₁₇ H ₁₃ N ₃ O ₃	307	3253	1643	–	1726	1659	–	–	–
CoL	C ₃₄ H ₂₄ N ₆ O ₆ Co	672	–	1602	1662	1714	–	1744	561	495
NiL	C ₃₄ H ₂₄ N ₆ O ₆ Ni	671	–	1603	1665	1710	–	1731	564	499
CuL	C ₃₄ H ₂₄ N ₆ O ₆ Cu	676	–	1599	1653	1702	–	1717	579	496
ZnL	C ₃₄ H ₂₄ N ₆ O ₆ Zn	678	–	1635	1653	1686	–	1730	566	497
CdL	C ₃₄ H ₂₄ N ₆ O ₆ Cd	725	–	1638	1657	1707	–	1722	577	498

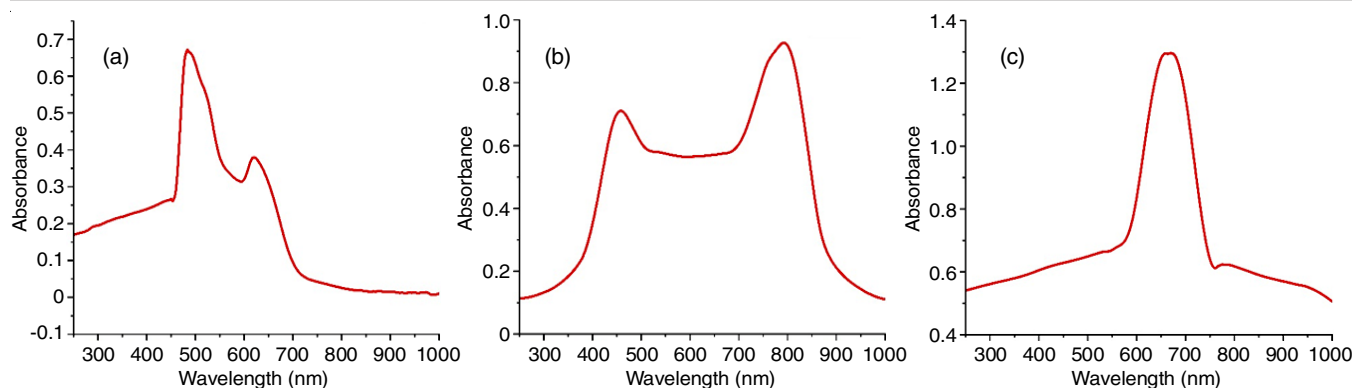


Fig. 1. UV-visible spectra of (a) CoL, (b) NiL and (c) CuL complex

positive, indicating a strong covalent character between the ligand (L) and its metal-complexes [45].

¹H NMR spectral studies: Two singlets were visible in the ¹H NMR spectrum of Schiff base ligand, one at 11.0 ppm (s, 1H, NH) and the other at 2.5 ppm (s, 3H, CH₃). The ligand, which is present in the range of 7.3-9.0 ppm, contains nine aromatic protons. The singlet at 9.0 ppm (s, 1H, ArH) verifies that a proton is present in the nitrogen-adjacent pyridinyl ring. Eight protons are left, of which three appear as triplets between 7.68 and 7.39 ppm (3t, 3H, ArH), four appear as doublets between 8.68 and 7.67 ppm (4d, 4H, ArH) and the final proton signals at δ 7.56 ppm (s, 1H, ArH). Comparatively, the disappearance of the amine -NH signal at 11.0 ppm (s) is the most significant change in the Zn(II) and Cd(II) complexes.

Mass spectral studies: The molecular ion peaks [M+1] at m/z 308 and 309 (100%, 16%) are observed, with the former being the base peak, corresponding to the ligand's molecular weight, as evidenced in the mass spectrum of ligand. A peak at m/z 185, 186 resulted from the fragmentation of CH_3 and $\text{C}_6\text{H}_4\text{NO}$ results in this molecular ion. As a result, the fragmentation pattern of ligand matches that of its structure.

The Co(II) complex mass spectrum offered strong support for the chemical formula $[\text{Co}(\text{C}_{17}\text{H}_{12}\text{N}_3\text{O}_3)_2]$ (m.w. 671.53). The Co(II) complex a peak of molecular ions was identified at m/z 671, 673, which is the same as its molecular weight. The loss of C_4H_4 caused this molecular ion to fragment and CH_3 molecules produced a peak detected at m/z 601, 603. The loss of $\text{C}_5\text{H}_5\text{N}$ group also resulted in a peak at m/z 526 and 528. Moreover, the complex was broken up by the loss of the $\text{C}_5\text{H}_5\text{N}$ group, which produced the peak detected at m/z 594 and 596. A peak at m/z 567, 569 was obtained due to the loss of C_2H_2 molecule and further loss of CH_3 , C_2H_2 and $\text{C}_5\text{H}_5\text{N}$ molecules resulted in peaks measured at m/z 543, 545 and 488, 490. The signals at m/z 307 and 309 confirm that the complex experienced fragmentation due to the loss of a ligand. As a result,

the Co(II) complex fragmentation pattern is compatible with the suggested structure.

Ni(II) complex mass spectrum offered strong support for the chemical formula $[\text{Ni}(\text{C}_{17}\text{H}_{12}\text{N}_3\text{O}_3)_2]$ (*m.w.* 671.29). The Ni(II) complex a peak of molecular ions were identified at m/z 671, 673 equal to complex molecular weight. The loss of C_4H_4 caused this molecular ion to fragment and CH_3 molecules produced a peak at m/z 601, 603. The complex was fragmented due to the loss of $\text{C}_5\text{H}_5\text{N}$ group, which produced the peak at m/z 594 and 596. Further, a peak at m/z 567, 569 was obtained as a result of the loss of the C_2H_2 molecule. Having lost molecules of the CH_3 , C_2H_2 and $\text{C}_5\text{H}_5\text{N}$ again, this produced a peak that was measured at m/z 543, 545 and 488,490. The signal at m/z 307, 309 confirms that the complex lost a ligand molecule.

The Cu(II) complex mass spectrum also offered support for the chemical formula $[\text{Cu}(\text{C}_{17}\text{H}_{12}\text{N}_3\text{O}_3)_2]$ (*m.w.* 676.14). The Cu(II) complex a peak of molecular ions were identified at m/z 676, 678, which is the same as its molecular weight. The loss of C_4H_4 caused this molecular ion to fragment and CH_3 molecules produced a peak at m/z 606, 608. The complex also experienced fragmentation due to the loss of $\text{C}_5\text{H}_5\text{N}$ group, which produced a peak at m/z 599, 601. Further, a peak at m/z 572, 574 was obtained due to the loss of the C_2H_2 molecule. After the loss of CH_3 , C_2H_2 and $\text{C}_5\text{H}_5\text{N}$ molecules, a peak was observed at m/z 549, 551 and 493, 495. The complex also went further fragmented by loss of ligand, as indicated by the signal at m/z 307 and 309. This indicates the proposed structure of Cu(II) complex and represented is in consistent fragmentation pattern of the Cu(II) complex.

For Zn(II) complex, a peak of molecular ions was identified at m/z 677, 679, which indicate its molecular weight. A peak at m/z 486 was observed, followed by m/z 488. The molecular ion underwent fragmentation resulting in the loss of $2C_2H_2$, N_2 and C_6H_4NO molecules. Furthermore, there is a peak observed at m/z 448, 450, 408, and 410, respectively, attributed

TABLE-3
ELECTRONIC SPECTRUM OF Co(II), Ni(II) AND Cu(II) METAL COMPLEXES' LIGAND FIELD PARAMETERS

[illegible]

to the loss of the C_2H_2 , CH_3 and C_5H_4N groups. The signal at m/z 307, 309 confirms that the complex has also undergone fragmentation by loss of ligand. As a result, Zn(II) complex mass spectrum fragmentation pattern is consistent with the framework that has been proposed for it.

The hypothesized structure of complexes is shown in Fig. 2 and is based on the analytical, physical and spectral data.

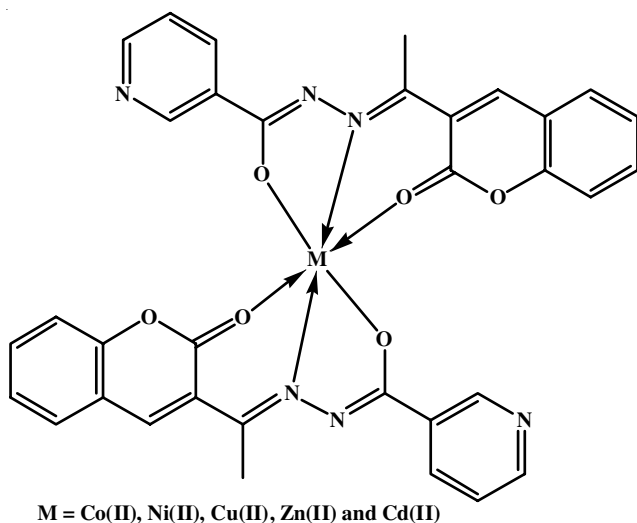


Fig. 2. Proposed structure of metal(II) complexes

In the mass spectrum of Cd(II) complex, a molecular ion peak was identified at m/z 725, 727, similar to Cd(II) complex empirical weight. The fragmentation of this molecular ion occurred due to the loss of molecules $2C_2H_2$, N_2 and C_6H_4NO , leading to the appearance of peaks at m/z 534 and 536. The peaks were seen at m/z 496, 498 and 456, 458, respectively, due to the loss of the C_2H_2 , CH_3 and C_5H_4N groups. The signal at m/z 307, 309 confirms that the complex also fragmented by the loss of one ligand.

Biological activity

In vitro antimicrobial activities: Using the conventional antibiotics ampicillin (AMP), ciprofloxacin (CIP), fluconazole

(FLZ) and itraconazole (ITZ), the new Schiff base ligand and its metal(II) complexes were tested for *in vitro* antibacterial activity. When tested against *E. coli*, *P. aeruginosa* and *B. subtilis*, the ligand shown good antibacterial activity (MIC 16 $\mu\text{g/mL}$) and moderate efficacy against *S. aureus* at (MIC 64 $\mu\text{g/mL}$). With good activity against *B. subtilis* (MIC 2 $\mu\text{g/mL}$), *S. aureus* (MIC 8 $\mu\text{g/mL}$), *E. coli* (MIC 8 $\mu\text{g/mL}$) and *P. aeruginosa* (MIC 4 $\mu\text{g/mL}$), the Cd(II) complex is observed to be an excellent physiologically active complex (Table-4). With MICs of 4 and 2 $\mu\text{g/mL}$ for *E. coli* and *P. aeruginosa*, respectively and 8 and 16 $\mu\text{g/mL}$ for *B. subtilis* and *S. aureus*, respectively. The Zn(II) complex has shown excellent activity against both bacteria. All of the bacteria were moderately responsive to Co(II), Ni(II) and Cu(II) complexes (MIC 32-256 $\mu\text{g/mL}$).

Both fungal strains exhibited a moderate level of resistance to the antifungal properties of Schiff base ligand, with a minimum inhibitory concentration of 128 $\mu\text{g/mL}$. Excellent activity for *C. albicans* (MIC 8 $\mu\text{g/mL}$) and moderate activity with *A. niger* (MIC 32 $\mu\text{g/mL}$) were displayed by Cd(II) complex. The Co(II) and Zn(II) complexes exhibit moderate efficacy against *A. niger* (MIC 64 $\mu\text{g/mL}$) and significant activity against *C. albicans* (MIC 32 $\mu\text{g/mL}$). All of the fungal strains were moderately resistant to the Ni(II) and Cu(II) complexes (MIC 64 and 128 $\mu\text{g/mL}$) (Table-4).

It was found that, in contrast to Schiff base ligand, the metal(II) complexes showed increased activity. Results showed that the coordination of metal ions with the ONO donor atoms in the ligand was improved by this approach. It is observed that the chelation enhances the ligand to act as more potent bactericidal and fungicidal agents [47]. The enhancement of antimicrobial activity may be rationalized by the fact that the ligand mainly has an azomethine ($C=N$) bond. Further in metal(II) complexes, the metal ions constructive charge is partially distributed with the hetero donor atoms (N and O) of the ligand and there may be experience π -electron delocalization over the whole chelating system [38,48]. This enhances the lipophilic characteristics of the metal chelates, allowing them to block the metal binding sites in enzymes produced by microbes by diffusing

TABLE-4
ANTIMICROBIAL ACTIVITY DATA OF NOVEL SCHIFF BASE LIGAND AND ITS METAL COMPLEXES

Compound	Concentration ($\mu\text{g/mL}$)											
	Bacteria								Fungi			
	Gram-positive				Gram-negative				Yeast		Filamentous	
	<i>B. subtilis</i>		<i>S. aureus</i>		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>C. albicans</i>		<i>A. niger</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
L	16	128	64	ND	16	256	16	128	128	ND	256	ND
CoL	32	ND	64	ND	32	256	32	ND	32	256	64	ND
NiL	16	256	32	256	16	256	8	128	64	ND	128	ND
CuL	16	128	32	256	64	ND	32	256	64	ND	128	ND
ZnL	8	64	16	128	4	64	2	32	32	256	64	ND
CdL	2	32	8	128	8	128	4	64	8	128	32	ND
AMP	4	16	16	256	—	—	—	—	—	—	—	—
CIP	—	—	—	—	4	16	1	256	—	—	—	—
FLZ	—	—	—	—	—	—	—	—	16	256	—	—
ITR	—	—	—	—	—	—	—	—	—	—	8	ND

ND: Not determined

through the lipid layer of bacterial cell membranes [38,49-51].

Antioxidant activity: According to the results obtained from DPPH and ABTS⁺ activities, the Cu(II) and Zn(II) complexes had good antioxidant properties when compared to ascorbic acid at all of the varied concentrations from 50-250 µg/mL. In comparison, Co(II), Ni(II) and Cd(II) complexes had moderate level of activity. The Cd(II) complex exhibited negligible efficacy when compared to standard drug (Fig. 3a-b).

In vitro anti-tubercular activity: The Cd(II) complex, with a minimum inhibitory concentration (MIC) of 16 µg/mL, exhibits the highest activity and also comparable to that of the standard drug ciprofloxacin against *M. tuberculosis* strain H37Rv.

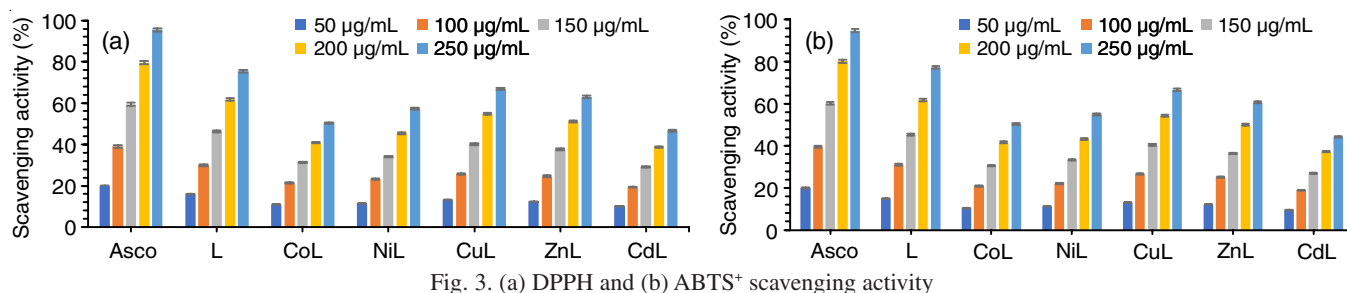


Fig. 3. (a) DPPH and (b) ABTS⁺ scavenging activity

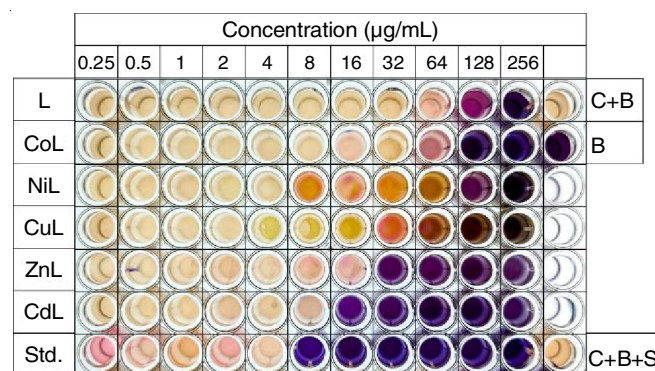


Fig. 4. Results of MIC of the newly synthetic substances in addition to the usual medication were determined by resazurin based micro-broth dilution method against *Mycobacterium tuberculosis* strain H37Rv

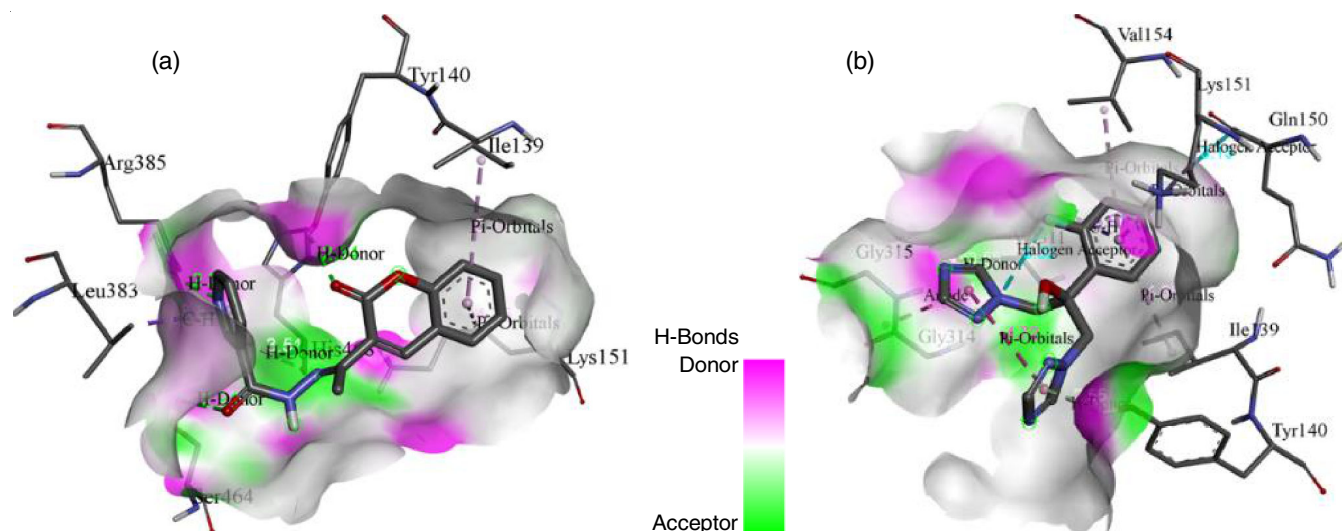
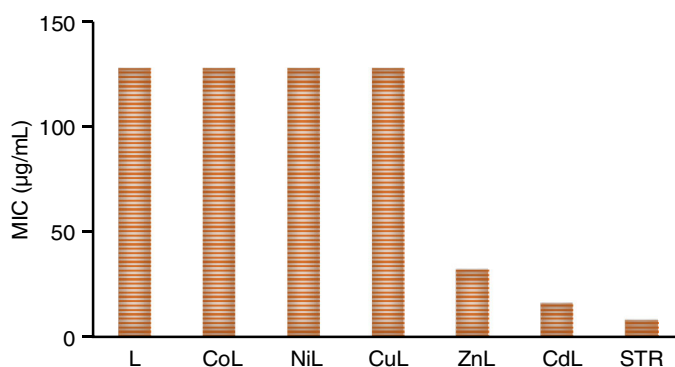


Fig. 5. The common medication fluconazole and the newly created ligand L₁ have the lowest binding energies at the active site of the enzyme lanosterol 14α-demethylase (4WMZ)

Whereas the Co(II), Ni(II), Cu(II) and Zn(II) complexes observed moderate anti-TB activity at MIC 32-128 µg/mL. At 128 µg/mL, the Schiff base ligand also demonstrated moderate anti-TB action (Fig. 4).

Molecular docking: Drug-receptor interactions were accurately shown by docking experiments, which identified nine possible interactions between each chemical and the protein. The BIOVIA Discovery studio visualizer shows the optimal interaction with the lowest binding energy. The information about the potential interactions including binding energy, interaction type, bond distance and bonding style. Schiff base ligand (L) produces four hydrophobic bonds—one pi-sigma, one pi-alkyl and three conventional—four hydrogen bonds, one carbon-

hydrogen link and three hydrophobic bonds with a binding energy of -8.7 Kcal/mol (Fig. 5a). As opposed to the typical drug fluconazole, which has a binding energy of -7.1 Kcal/mol and produces two hydrogen bonds, two halogens and five hydrophobic contacts (Fig. 5b).

Conclusion

A newly Schiff base ligand, N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)nicotinothiazide and its Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes was successfully synthesized and characterized. The synthesized metal(II) complexes exhibit the octahedral geometry and the ligand behaves as a tridentate chelate (ONO donor) surrounded to the metal ions, according to the physico-chemical and spectroscopic techniques. The Schiff base ligand is found to be biologically most effective against all strains of microorganisms and as the complex forms, this activity increases. The antimicrobial activity investigation showed that complex formation increased the activity of Schiff base ligand. The Cd(II) complex (MIC 2 and 8 µg/mL) against the studied microorganisms may be considered as promising antimicrobial agents. Furthermore, *M. tuberculosis* was effectively inhibited by the Cd(II) metal complex. Excellent anti-oxidant activity were displayed by the Co(II), Ni(II) and Cu(II) complexes.

ACKNOWLEDGEMENTS

The authors are grateful to The Chairman of Chemistry Department, Gulbarga University, Kalaburagi, for providing the research facilities. The analytical and spectral data provided by SAIF, Punjab University, Central University of Karnataka and Shri Krishnadevaraya University, Ballari, India are also appreciated. The authors also acknowledge Dr. Praveen Kumar S.K., Department of Biochemistry, Karnataka University, Dharwad, for other biological studies and the Central Research Laboratory, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, India, for conducting the anti-tuberculosis (TB) activities.

CONFLICT OF INTEREST

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

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