



Synthesis, Spectral, Thermal Studies and Antimicrobial Evaluation of Transition Metal Complexes with Novel Schiff base Ligand

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ABSTRACT

A novel coumarin Schiff base "(E)-7-hydroxy-4-methyl-8-(1-(1-(naphthalen-2-yl) ethylimino) ethyl)-2H-chromen-2-one" [HOMNEIEC] ligand was synthesized by the condensation of "8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one" [AHMC] with "1-(naphthalen-1-yl) ethylamine" [NEA]. The transition metal (II) ions complexes with this ligand were synthesized by a common method. The synthesized ligand and metal (II) complexes were studied using spectral (¹H NMR, FTIR, Mass Spectrometry) and thermal (TGA) techniques for their structures. The ¹H NMR and FTIR spectra of ligand confirm the formation of azomethine bond. The FTIR spectral data validated the formation of coordinate bond through phenolic oxygen on coumarin ring and nitrogen of azomethine with the metal ions. The m/z values in mass spectrum of ligand and its metal complexes were in agreement with their theoretical values of molecular/formula weights. The TGA thermograms suggested there are of two coordinated/lattice H₂O molecules in each of the complex. The ligand and metal complexes were evaluated for their *in vitro* antimicrobial activities using broth microdilution method using DMSO solvent/diluent against *Escherichia coli* and *Pseudomonas aeruginosa* a Gram-negative bacterial strain, *Staphylococcus aureus* and *Streptococcus pyogenes* a Gram-positive bacterial strain, and *Candida albicans*, *Aspergillus clavatus* and *Aspergillus niger* a fungal strain. The metal complexes were found to have enhanced antimicrobial activities compare to the Schiff base ligand.

Keywords: Coumarin Schiff base, Metal complexes, Spectroscopy, Thermogravimetry, Broth dilution method.

INTRODUCTION

The oxygen and nitrogen donor (O, N donor) ligands have a crucial role in the formation of transition metal coordination complexes. A good deal of research was carried out on synthesis, spectral, thermal studies and antimicrobial evaluation of metal

complexes with these ligands. The stability and biological activities of these complexes were due to chelating property of the ligand¹⁻⁷.

In this case the best suitable ligands known are heterocyclic compounds. An important class of organic heterocyclic compounds, acetyl/formyl



hydroxy coumarin derivatives and its Schiff bases with aliphatic/aromatic primary amines, have been extensively studied for their structural, biological and photochemical properties. These ligands possess excellent ability to coordinate with the transition metal ions due to formation of chelate through hydroxyl oxygen and azomethine nitrogen⁶⁻¹⁶.

The metal complexes of coumarin Schiff base ligands with Cu(II), Zn(II), Co(II), and Ni(II) ions will have a better choice for such studies. Since the complexes with these metal (II) ions can be readily prepared^{17,18} and also play an important role in enhancing their biological activities.

The review of recent year's literature reveals that Schiff bases of coumarin derivatives with aromatic amines show antibacterial and antifungal¹⁹ activities. A broad array of antibacterial activity²⁰ of such Schiff bases have been investigated. However, very few studies were reported about the Schiff base ligands containing both coumarin²¹⁻²⁴ and naphthalene²³ moieties and its metal (II) complexes. These ligands are polyfunctional molecules consist of oxygen and nitrogen bearing functional groups which can trap the transition metal ions and form metal chelates. Such metal (II) chelates may have higher antimicrobial activities than the Schiff base ligands. This led us to synthesise of new Schiff base ligand containing both coumarin and naphthalene moieties with oxygen and nitrogen donor groups at appropriate positions and its transition metal (II) ion complexes. In this article, synthesis, spectral, thermal studies and antimicrobial evaluation of Schiff base ligand and its bivalent transition metal ions complexes are reported for the first time. This molecule consists of hydroxyl (-OH) and azomethine (>C=N-) groups, which act as a suitable chelating ligand and its metal complexes have moderate to high antimicrobial activities.

EXPERIMENTAL

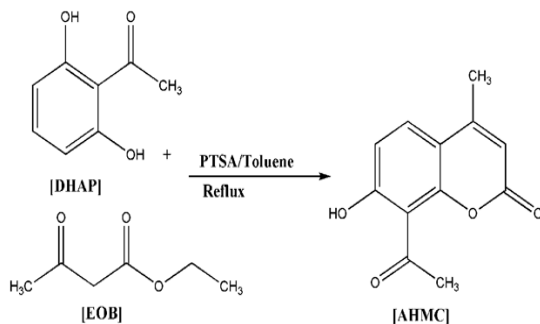
The reagents, solvents and chemicals used in the experiments were obtained from TCI Chemicals, Loba Chemie, S.D. Fine Chem Ltd. and were utilized as procured. All the solvents required of analytical grade were used and were dried according to the standard procedures in Vogel's Textbook of Practical Organic Chemistry²⁵.

All metal (II) chloride salts were used for metal complexes synthesis. All reactions were stirred magnetically and thin layer chromatography (TLC) Aluminium silica gel coated plates were used to monitored the progress of the reactions and the plates were visualized under Ultraviolet light. The Decomposition points (D.P.)/Melting points (M.P.) were measured by an open capillary method. The metal (II) ions content and metal to ligand ratio of metal complexes were analysed volumetrically by the reported method in Vogel's Textbook of Quantitative Chemical Analysis²⁶.

FT-IR spectrophotometer in KBr, Bruker Germany, Model No. 3000 Hyperion Microscope with Vertex 80 FTIR System were used to record the IR spectra. NMR spectrometer 600 MHz, JEOL, Japan, ECZR Series, CDCl₃ as a solvent and TMS as a reference were used to record ¹H NMR spectra. Mass spectra were recorded on a Waters Single COD, Model No. Waters-3100. Perkins Elmer, Model No. TGA-4000 were used to record TGA thermograms. The *in vitro* antimicrobial evaluation was performed by broth microdilution method and the organisms/strains are listed in Table 3 and Table 4.

Synthesis of 8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one [AHMC]

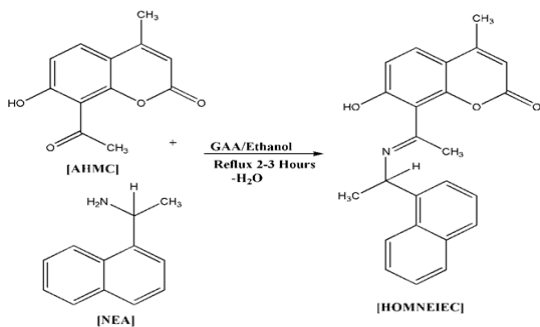
Synthesis of "8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one" [AHMC] was performed from 2, 6-dihydroxyacetophenone [DHAP] and ethyl 3-oxobutanoate [EOB] as per the reported method by Hejchman *et al.*, (2008)^{27a} and Furuta *et al.*, (2004)^{27b} (Scheme 1). Heated a mixture of 2, 6-dihydroxyacetophenone [DHAP] (1.0 mmol) and ethyl acetoacetate [EAA] (1.2 mmol) with catalytic amount of p-toluene sulfonic acid [PTSA] in 10 mL toluene and refluxed for 8 h with azeotropic removal of water and ethanol by the use of a Dean-Stark trap. The progress of the reaction was monitored using TLC by 50% Ethyl Acetate/Hexane solvent mixture. The reaction mixture was left overnight. Next day ice-water was added to the mixture. The mixture was stirred for 45 min filtered of and dried the precipitate. Product obtained was purified by recrystallization in hot methanol. Appearance off white and melting point 170–172°C. Yield of the reaction was about 50% with respect to 2,6-dihydroxyacetophenone.



Scheme 1. Reaction Scheme for Synthesis of [AHMC]

Synthesis of Schiff base (E)-7-hydroxy-4-methyl-8-(1-(1-(naphthalen-2-yl) ethylimino) ethyl)-2H-chromen-2-one [HOMNEIEC] Ligand

Synthesis of a novel Schiff base (E)-7-hydroxy-4-methyl-8-(1-(1-(naphthalen-2-yl) ethylimino) ethyl)-2H-chromen-2-one [HOMNEIEC] ligand was achieved by condensation of "8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one" [AHMC] and "1-(naphthalen-1-yl) ethylamine" [NEA] as per the method by Aazam *et al.*, (2012)¹⁰ (Scheme 2). A clear solution of (S)-1-(naphthalen-1-yl) ethane amine [NEA] (1.1 mmol) in 5 mL ethanol was added to a warm solution of 8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one [AHMC] (1.0 mmol) in 10 mL ethanol with catalytic amount of glacial acetic acid. The resulting mixture was refluxed for 2-3 h and progress of the reaction was monitored using TLC by 50% Ethyl Acetate/Hexane solvent combination. The refluxed mixture then kept overnight. Yellow product was precipitated, filtered of and washed with cold ethanol. Product was purified by recrystallization from ethanol, dried in a vacuum desiccator. Appearance yellow colour solid and melting 206–208°C. Soluble in DMF, DMSO and Chloroform. Yield of the reaction was about 60% with respect to 8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one.



Scheme 2. Reaction Scheme for Synthesis of [HOMNEIEC]

¹H NMR Spectrum of Schiff base:

(δ ppm, CDCl_3/TMS) 2.37 (s, 3H, Ar- CH_3) a singlet for methyl group attached to coumarin ring at 4th position, 1.90 (d, 3H, $>\text{CH}-\text{CH}_3$) a doublet for methyl group due to adjacent carbon bearing one hydrogen atom, 1.61 (q, 1H, $>\text{CH}-\text{CH}_3$) this -CH- give quartet due to three hydrogens on adjacent carbon, 2.68 (s, 3H, $=\text{N}-\text{C}-\text{CH}_3$) methyl group attached to azomethine carbon give singlet, (Fig. 1a), 5.95 (s, 1H, Ar-OH) hydroxyl group on coumarin ring at 7th position, 7.26 (s, 1H, $=\text{C}-\text{H}$) aromatic hydrogen at 3rd position on coumarin moiety, and 7.90-7.95 (d, 1H), 8.05-8.10 (d, 1H) are two aromatic hydrogens on coumarin ring at 5th and 6th positions respectively and 7.45-7.65 (m, 2H), 7.80-7.84 (m, 5H), all of these multiplet are for hydrogens of naphthalene moiety (Figure 1b).

FTIR Spectrum of Schiff base: ν

(KBr, cm^{-1}): 1730 (lactonyl, $-\text{O}-\text{C}=\text{O}$) this IR peak is for cyclic ester functional group which is the fundamental part of coumarin, 1592 and 1627 peaks (azomethine, $>\text{C}=\text{N}-$) for Schiff base functional group in resonance with the pi electrons of aromatic rings, 1372, 1434, 1508 (aromatic, $-\text{C}=\text{C}-$) for aromatic rings, 1238 ($-\text{C}-\text{O}-$) this peak represents $-\text{C}-\text{O}-$ bond between carbon of coumarin ring and oxygen of hydroxyl group, 1175 ($-\text{C}-\text{N}-$) assigned to the carbon of naphthalene ring and nitrogen of azomethine, 1085 ($-\text{C}-\text{C}-$) carbon-carbon single bonds, 3432 (hydroxyl, $-\text{O}-\text{H}$) a prominent peak in this region of IR spectrum observed for hydroxyl stretching vibration, 2978 (aliphatic C-H) stretching band observed due to the three $-\text{CH}_3$ and one $-\text{CH}-$ groups, 3048 (aromatic C-H) there are ten aromatic hydrogens present including both coumarin and naphthalene moieties given this band for C-H stretching, 446-924 (aromatic C-H) bending vibrations (Figure 2).

Mass Spectrum of Schiff Base: m/z :

($M+H$) = 372.5, 284.7, 451.7, 452.1 (Fig. 3). m/z of 372.5 represents the base peak. The molecule (M) with H^+ give $[M+H]^+$ peak. It formed due to the presence of heteroatoms in the molecule. The calculated molecular weight of ligand was 371.45 g/mol. As per the m/z values in the mass spectrum, $m/z = 372.5$ which represents ($M+H$) is in agreement with the calculated value. This supports the molecular weight of ligand is equal to 371.45 g/mol.

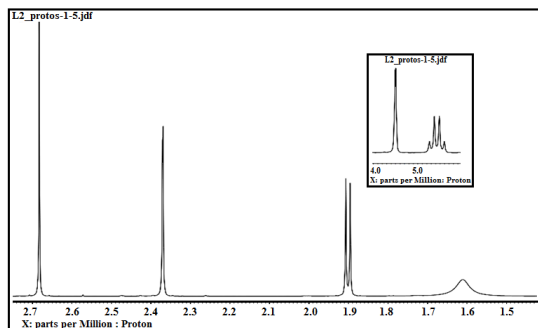
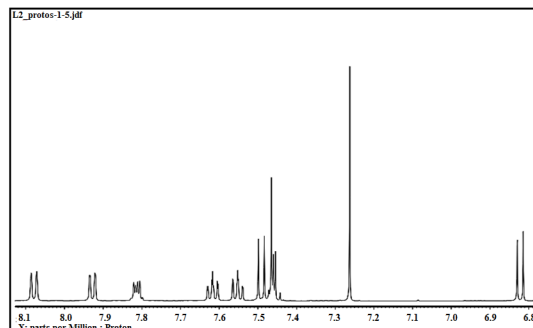
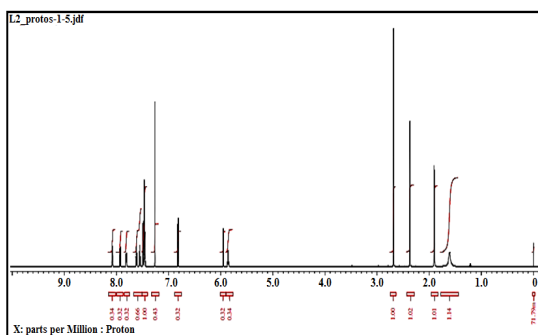
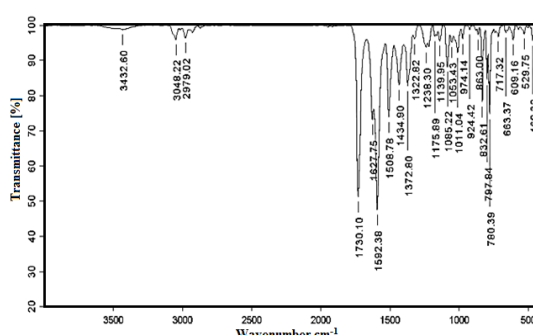
Fig. 1a. ¹HNMR Spectrum of Schiff base [HOMNEIEC]Fig. 1b. ¹HNMR Spectrum of Schiff base [HOMNEIEC]Fig. 1c. ¹HNMR Spectrum of Schiff base [HOMNEIEC]

Fig. 2. IR Spectrum of Schiff base [HOMNEIEC]

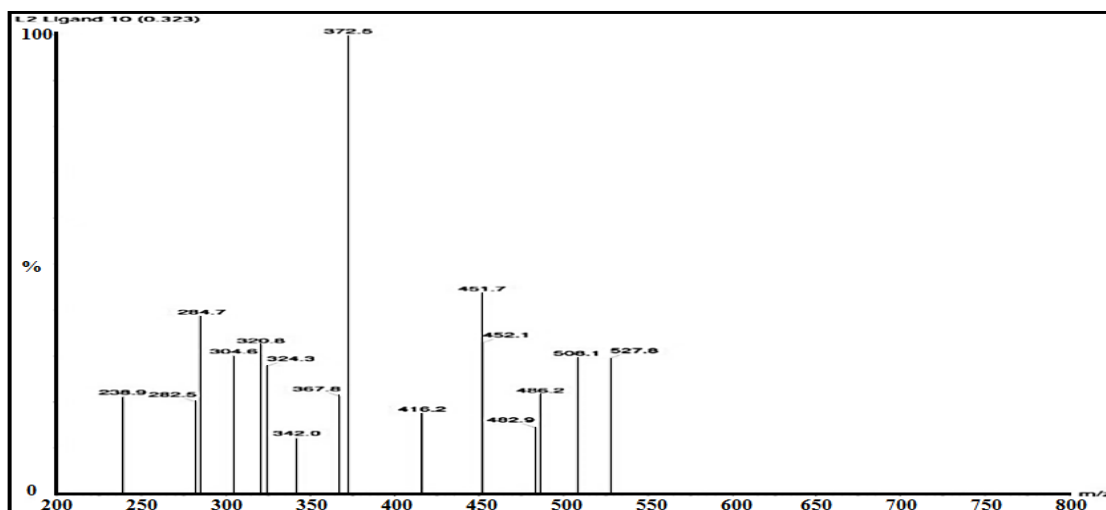


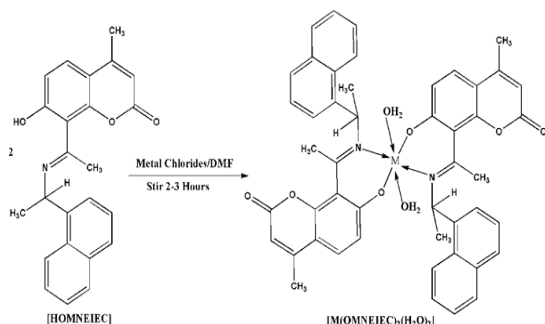
Fig. 3. Mass Spectrum of Schiff base [HOMNEIEC]

Synthesis of Metal (II) Complexes

The metal (II) complexes with Schiff base (E)-7-hydroxy-4-methyl-8-(1-((1-(naphthalen-1-yl) ethyl) imino) ethyl)-2H-chromen-2-one [HOMNEIEC] ligand were synthesised by a common method reported by Adhao *et al.*, (in press)³⁴ (Scheme 3). To a clear solution of [HOMNEIEC] (0.5 mmol) in 15 mL dimethylformamide (DMF) was added a respective metal (II) chloride salt (1.0 mmol) separately with

catalytic amount of aqueous ammonia (For the purpose to maintain basicity) at 0-5°C. Then the resulting mixture was moved to room temperature slowly and stirred for 2-3 hours. The progress of the reaction was checked using TLC by 5% methylene dichloride/methanol solvent mixture. The reaction mixture was diluted with diethyl ether and stirred for 2 h to obtain the solid product. The obtained solid was filtered on Whatman filter paper and washed

with diethyl ether and dried for 30 min under vacuum. The yields of the reaction were between 70% to 75% with respect to the Schiff base [HOMNEIEC]. Coloured solid metal complexes were obtained with M.P./D.P. between 180°C to 190°C and found soluble in DMF and DMSO.



Scheme 3. Reaction Scheme for Synthesis of Metal (II) Complexes with [HOMNEIEC] Where, M = Cu(II), Ni(II), Co(II) and Zn(II)

Physical and Spectral Studies of Metal Complexes

Co(II) ion Complex with Schiff base

[HOMNEIEC]: Yield 70%, black colour solid, M.P./D.P. 184°C, M.W./F.W. 835.83 g/mol, soluble in DMF and DMSO. IR Spectrum: (ν , cm^{-1}): (Fig. 4) 1730, (lactonyl, -O-C=O) cyclic ester part of coumarin ring, 1577 (azomethine, >C=N-) this group frequency shifted to lower side indicate the coordination bond formation with nitrogen, 1398 (aromatic, -C=C-), 1288 (phenolic, -C-O-), 1226 (-C-N-), 1090 (aliphatic, -C-C-), 2900 (aliphatic, -C-H), 3000 (aromatic, C-H), 3155 (Broad) for H_2O lattice/coordinated hydrogen bonded, 820 with shoulder peak (coordinated water molecules with metal ion). Mass Spectrum: (Fig. 5) m/z : (L+H)=372.5, this peak is due to ligand. (M+H)=836.7, 836.9 are m/z for metal complex. The metal complex and H^+ combined to give a peak of one unit more than the complex mass. The calculated expected molecular/ formula weight of complex was 835.83 g/mol. This is in agreement with the m/z value obtained in the mass spectrum for the metal complex.

Ni(II) ion Complex with Schiff base

[HOMNEIEC]: Yield 75%, brick red colour solid, M.P./D.P. 185°C, M.W./F.W. 835.59 g/mol, soluble in DMF and DMSO. IR Spectrum: (ν , cm^{-1}): 1721 (lactonyl -O-C=O) cyclic ester, 1593 (azomethine >C=N-) imino, 1391 (aromatic -C=C-), 1282 (phenolic -C-O-), 1218 (-C-N-), 1087 (aliphatic -C-C-), 2900 (aliphatic -C-H stretching), 3000 and 3043 (aromatic -C-H stretching), 3156-3322 (Broad) H_2O lattice/coordinated hydrogen bonded, 826 with small shoulder peak (coordinated water molecules with metal ion). Mass Spectrum: m/z (L-H) = 370.4, this peak is for ligand. (M-2) = 833.5, (M-H) = 834.5 are m/z for metal complex. This supports the calculated formula weight for the complex is equal to 835.59 g/mol.

Cu(II) ion Complex with Schiff Base

[HOMNEIEC]: Yield 72%, sap green colour solid, M.P./D.P. 188°C, M.W./F.W. 840.44 g/mol, soluble in DMF and DMSO. IR Spectrum: (ν , cm^{-1}): 1728 (lactonyl -O-C=O) cyclic ester, 1590 (azomethine >C=N-), 1392 (aromatic -C=C-), 1253 (phenolic -C-O-), 1222 (-C-N-), 1086 (aliphatic -C-C-), 2815 (aliphatic -C-H), 3041 (aromatic -C-H), 3223-3323 (Broad) H_2O lattice/coordinated hydrogen bonded, 818 & 838 (coordinated water molecules with metal ion). Mass Spectrum: m/z = (L+H) = 372.4, it represents ligand. (M+H) = 841.3 and 841.4 and (M+) = 840.3 are for the metal complex. The peak, m/z = 840.3 is almost equal to the calculated formula weight 840.44 g/mol of the metal complex.

Zn(II) ion Complex with Schiff base

[HOMNEIEC]: Yield 70%, buff white colour solid, M.P./D.P. 190°C, M.W./F.W. 842.29 g/mol, soluble in DMF and DMSO. IR Spectrum: (ν , cm^{-1}): 1730 (lactonyl -O-C=O), 1594 (azomethine >C=N-), 1392 (aromatic -C=C-), 1242 (phenolic -C-O-), 1166 (aliphatic -C-C-), 3000-3100 (aromatic -C-H), 3233-3353 (Broad) H_2O lattice/coordinated hydrogen bonded, 663 with small shoulder peak (coordinated water molecules with metal ion). Mass Spectrum: m/z = (L+H) = 372.4, for ligand. (M+H) = 843.2, 843.9, are for the metal complex which is nearly in agreement with the formula weight of the metal complex 842.29 g/mol. Where L is ligand and M is metal complex.

Table 1: Physical Analysis of Ligand and Metal (II) Complexes

Ligand/Complexes	M.W./F.W.g/mol	Colour	%Yield	M.P./D.P.°C	At. Wt. of Metal	%Metal Content Observed (Calculated)
HL2:C ₂₄ H ₂₁ NO ₃	371.45	Yellow	60	208	-	-
[Co(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	835.83	Black	70	184	58.93	6.900 (7.050)
[Ni(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	835.59	Brick Red	75	185	58.69	6.850 (7.024)
[Cu(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	840.44	Sap Green	72	188	63.54	7.400 (7.560)
[Zn(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	842.29	Buff White	70	190	65.39	7.600 (7.763)

M. W.–Molecular Weight, F. W.–Formula Weight, M. P.–Melting Point, D. P.–Decomposition Point, At. Wt.–Atomic Weight

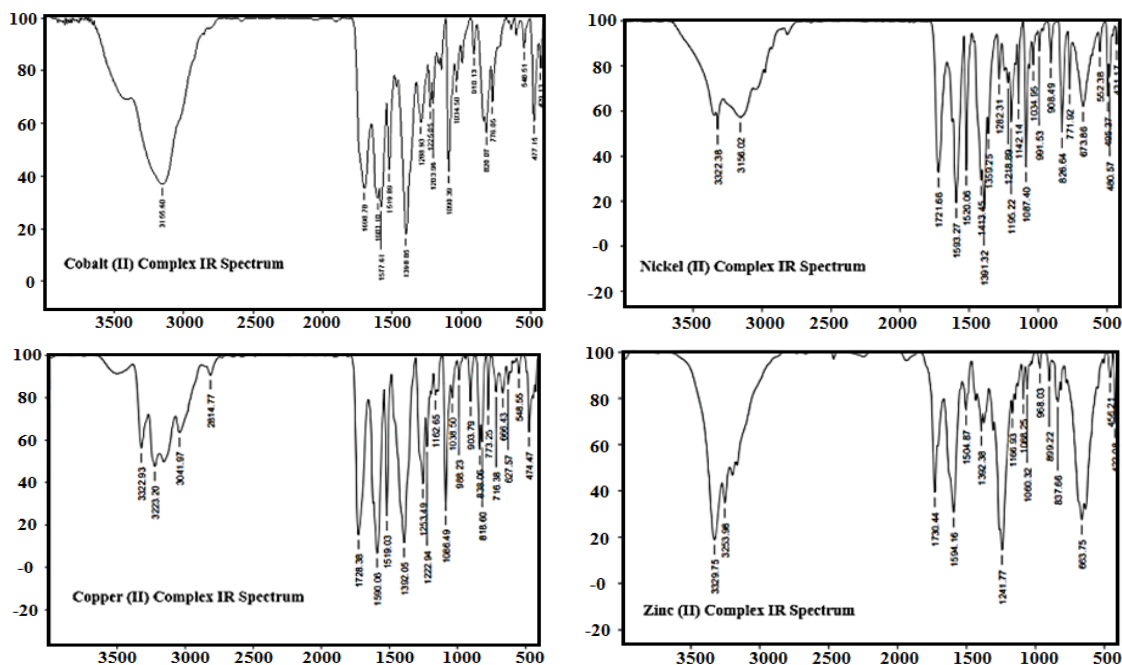


Fig. 4. IR Spectrum of Metal (II) Complex with [HOMNEIEC]

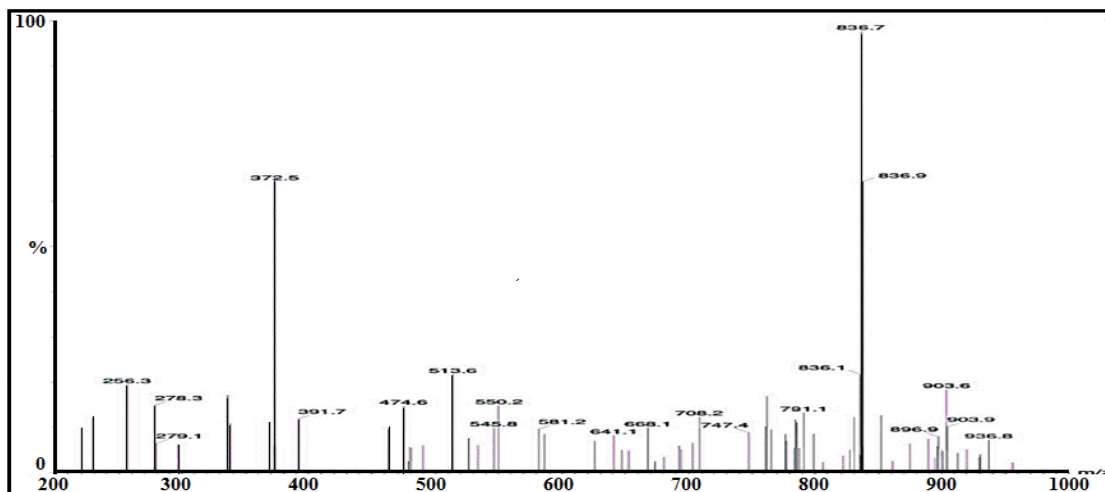


Fig. 5. Mass Spectrum of Co(II) Complex with [HOMNEIEC]

Thermal (TGA) Studies of Metal Complexes

The TGA thermograms were recorded and the % weight loss in each step was evaluated with respect to the heating rate of 20°C/min between

30°C to 900°C. The observed and calculated TGA data of %weight loss and residue remained is summarized in Table 2 for the metal complexes of [HOMNEIEC]/HL2 ligand.

Table 2: TGA Data of Metal Complexes

Formula of Complexes	% of Metal ion Observed (Calculated)	% of H ₂ O bserved (Calculated)	M.W./F.W. g/mol	Wt. of Sample (mg)	%Weight Loss 30-105°C	%Residue (Oxides) 105-917°C
[Co(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	7.443 (7.050)	5.562 (4.307)	835.83	4.765	5.562	84.700
[Ni(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	7.767 (7.024)	5.897 (4.308)	835.59	3.708	5.897	84.218
[Cu(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	6.795 (7.560)	6.098 (4.283)	840.44	3.742	6.098	84.540
[Zn(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	9.172 (7.763)	5.380 (4.274)	842.29	4.843	5.380	83.203

M.W.- Molecular Weight, F.W.- Formula Weight, Wt.- Weight

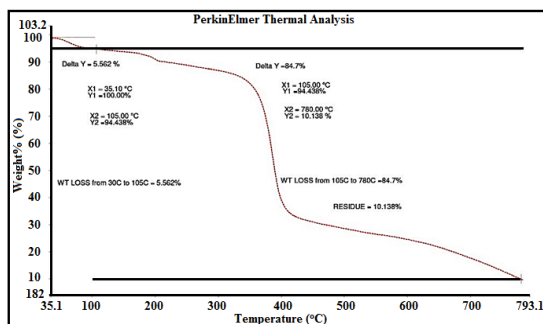


Fig. 6. TGA Thermogram of Co(II) Complex

Antimicrobial activities studies

The *in vitro* antimicrobial activities of Schiff base [HOMNEIEC]/HL2 and its transition metal (II) ions complexes were evaluated in DMSO medium/

diluent using the broth microdilution method²⁹⁻³¹. The MIC (Minimum Inhibitory Concentration) values in microgram per mL for the synthesized coumarin Schiff base ligand and its metal (II) ions complexes were determined against *E. coli* and *P. aeruginosa* a Gram-negative bacterial strain, *S. aureus* and *S. pyogenes* a Gram-positive bacterial strain, and *C. albicans*, *A. clavatus* and *A. niger* fungal strains listed in the Table 3 and Table 4 and comparisons in their activities shown in Fig. 7 and Fig. 8 respectively. Chloramphenicol (antibacterial) and griseofulvin (antifungal) drugs were used as reference for this antimicrobial evaluation.

Antibacterial Activity of Ligand and Metal Complexes

Table 3: MIC Values of Schiff Base [HOMNEIEC]/HL2 and Its Transition Metal Complexes

Test Compound/Complex	Test Organisms Minimal Inhibitory Concentration [MIC] (µg/mL)			
	Gram-negative		Gram-positive	
	<i>E. coli</i> (MTCC 443)	<i>P. aeruginosa</i> (MTCC 1688)	<i>S. aureus</i> (MTCC 96)	<i>S. pyogenes</i> (MTCC 442)
HL2: C ₂₄ H ₂₁ NO ₃	100	125	125	100
[Co(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	125	200	125	200
[Ni(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	100	125	100	200
[Cu(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	100	62.5	125	125
[Zn(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	62.5	100	100	100
Chloramphenicol	50	50	50	50

Table 4: MIC Values of Schiff Base [HOMNEIEC]/HL2 and Its Transition Metal Complexes

Test Compounds/Complexes	Test Organisms Minimal Inhibitory Concentration [MIC] (µg/mL)		
	<i>C. albicans</i> (MTCC 227)	<i>A. clavatus</i> (MTCC 1323)	<i>A. niger</i> (MTCC 282)
HL2: C ₂₄ H ₂₁ NO ₃	500	>1000	>1000
[Co(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	>1000	>1000	>1000
[Ni(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	500	>1000	>1000
[Cu(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	250	1000	500
[Zn(C ₂₄ H ₂₀ NO ₃) ₂ (H ₂ O) ₂]	500	1000	1000
Griseofulvin	500	100	100

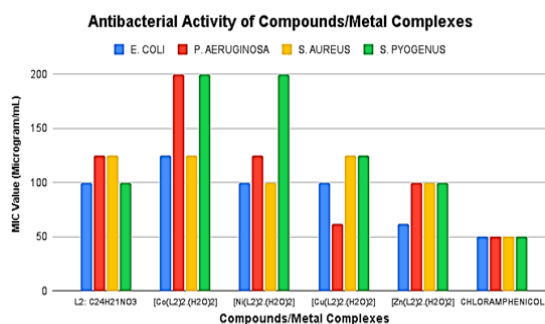


Fig. 7. Antibacterial Activity of [HOMNEIEC], HL2 and Metal (II) Complexes

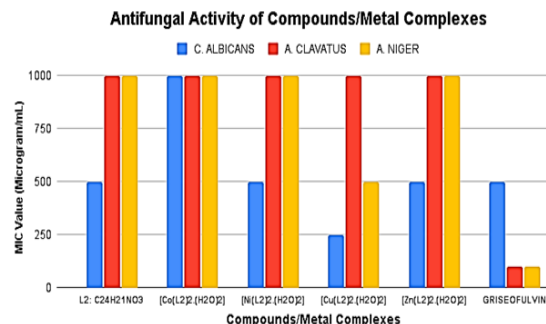


Fig. 8. Antifungal Activity of [HOMNEIEC], HL2 and Metal Complexes

RESULTS AND DISCUSSION

Synthesis: A novel Schiff base (*E*)-7-hydroxy-4-methyl-8-(1-(1-(naphthalen-2-yl)

ethylimino ethyl)-2*H*-chromen-2-one [HOMNEIEC] ligand was synthesized by condensation of "8-acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one" [AHMC] and "1-(naphthalen-1-yl) ethylamine" [NEA] (Scheme

2). "8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one" [AHMC] was synthesized as per the method reported in the literature (Scheme 1). The metal (II) ions complexes were synthesized by using a common method. The reactions of metal (II) chlorides of cobalt, nickel, copper and zinc separately with the Schiff base ligand were performed to obtain the metal complexes (Scheme 3). The synthesized ligand and its metal (II) ions complexes were studied for their structures by using physical, spectral and thermal methods.

¹H NMR Spectroscopy: The characteristic ¹H NMR signal for aliphatic -NH₂ (between 2.0-3.0 ppm)³³ does not appear in the ¹H NMR spectrum of ligand proving that the azomethine (>C=N-) bond successfully formed in place of amino (-NH₂). The signals for hydrogens of three -CH₃ groups, one >CH- group, one phenolic hydrogen and ten aromatic hydrogens were observed with their signature chemical shift values (δ in ppm) that justified the proposed structure of the Schiff base ligand.

FTIR Spectroscopy: The IR spectrum of [HOMNEIEC] consists of various bands representing -O-C=O (ester), >C=N- (azomethine), -C=C- (aromatic), -C-O-, -C-C-, -O-H (hydroxyl), -C-H (aliphatic C-H stretching), -C=C-H (aromatic C-H stretching), M-N (metal and nitrogen), M-O (metal and oxygen), and C-H bending vibrations. In the IR spectrum of ligand, the peak for amino (-NH₂) group does not appear indicating the formation of azomethine (>C=N-) group (Fig. 2). The presence of characteristic metal-oxygen and metal-nitrogen IR bands (400-600 cm⁻¹)^{10,35} indicate the formation of metal chelates and the ligand is oxygen and nitrogen donor. The characteristic peak for the hydroxyl (-O-H) group does not appear in the IR spectrum of metal complexes. It confirms that the hydrogen atom detached and metal ligand coordinate bond formation takes place through oxygen. The broad peak between 3200-3600 cm⁻¹ suggests presence of lattice/coordinated H₂O molecules associated with the metal (II) ions complexes. The peaks between 820-840 indicate the coordinated H₂O molecules with the metal ions (Figure 4).

Mass Spectrometry: The m/z values in the mass spectra of ligand and its metal complexes were found in concordance with the calculated values of molecular weight of ligand and the formula

weights of metal complexes. The m/z values obtained support the molecular weight of Schiff base (371.45 g/mol) and the molecular/formula weights of metal complexes (Table 1).

Thermogravimetry: The TGA studies showed that the percentage (%) of metal ions observed and calculated were found to be nearly equal (Table 2). The TGA thermograms of the metal complexes suggest that there are coordinated/lattice H₂O molecules associated with the metal complexes. The slightly higher observed percentage weight loss in the beginning may be due to the removal of non-coordinated part of ligand such as methyl group²⁸ along with water. It can be seen in the TGA thermograms that the decomposition of metal complexes started between 180°C to 200°C and the major loss in weight was observed between 400°C to 500°C. For Co(II) and Cu(II) ions complexes decomposition started from 180°C onwards and sharp loss in weight was observed at around 400°C and for Ni(II) and Zn(II) ions complexes the decomposition commenced from 200°C onwards and it was continued upto 500°C. From the decomposition pattern it is evident that the metal complexes are thermally stable at room temperature. All complexes more or less showed similar decomposition trends. First there was loss of water molecules between 30°C to 105°C, decomposition started at 180°C or 200°C and a major loss in weight was observed after 400°C or 500°C and the decomposition completed at and above 780°C.

In vitro Antibacterial and Antifungal Evaluation: *In vitro* antibacterial evaluation (Fig. 7) and *in vitro* antifungal evaluation (Fig. 8) of the synthesized coumarin Schiff base [HOMNEIEC]/HL2 and its metal (II) complexes show that the complexes are more active towards the microbes compared to the ligand. They have more toxicity toward the selected microbes than the ligand (Table 3 and Table 4). The MIC value for the Cu(II) ion complex against *P. aeruginosa* a Gram-negative bacterial strain was 62.5 µg/mL and the MIC value for the Zn(II) ion complex against *E. coli* a Gram-negative bacterial strain was 62.5 µg/mL. These values are nearer to the MIC values for the standard drug chloramphenicol (50 µg/mL). Chloramphenicol is a known antibiotic used in human medicine for treatment of infections due to methicillin sensitive *S. aureus* a Gram-positive bacterial strain. The MIC value for Cu(II) ion complex

against *C. albicans* a fungal strain was 250 µg/mL, which is lower than the MIC value for the standard drug *Griseofulvin* (500 µg/mL). The antifungal activity of Cu(II) ion complex was found to be two-fold more than *Griseofulvin* against *C. albicans* fungal strain. *Griseofulvin* is an antifungal agent used to treat fungal infections of the skin, hair, and nails. It acts by preventing the growth of fungi.

CONCLUSION

A bidentate (O, N- donor) Schiff base ligand [HOMNEIEC] containing both coumarin and naphthalene moieties and its metal(II) ions complexes, [M(OMNEIEC)₂(H₂O)₂] were synthesized successfully. The FTIR, ¹H NMR, Mass spectrometry, and TGA methods were used to confirm the structures of synthesized ligand (Scheme 2) and its metal(II) ions complexes (Scheme 3). The ligand was found coordinated through phenolic-oxygen (on coumarin ring) and nitrogen of azomethine group forming stable chelates with metal to ligand

ratio 1:2 and the metal complexes were found stable at ambient temperature. All of these studies and evaluation concluded that this oxygen and nitrogen donor Schiff base ligand and the selected metal(II) ions have formed stable metal complexes and the Copper(II) and Zinc(II) ions complexes found to have enhanced antimicrobial activities than the ligand.

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Conflict of interest

The Authors declares they have no conflict of interest.

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