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Synthesis, Characterization and Investigation of Biological Activities of Some New Zinc Complexes of Imidazo[4,5-f]1,10-phenanthroline Derivatives

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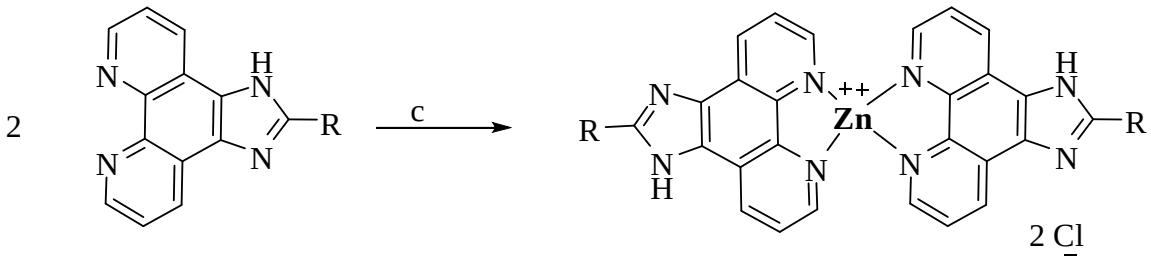
ABSTRACT

The paper presents the synthesis of new complexes of Zn(II) with imidazole-based ligands L1-L4 {L1=2-p-tolyl-(1H-imidazo[4,5-f][1,10] phenanthroline), L2=2-p-chloro-benzene-(1H-imidazo[4,5-f][1,10]phenanthroline), L3=2-(3-indole)-(1H-imidazo [4,5-f][1,10]phenanthroline), L4=2-(3-7-azaindole)-(1H-imidazo[4,5-f][1,10]phenanthroline)} obtained from multi-component reaction of 1,10-phenanthroline-5,6-dione, aromatic/heterocyclic aldehydes and ammonium acetate. The newly synthesized ligands and complexes were characterized by combination of elemental analysis, ^{1}H-NMR spectroscopy, ^{13}C-NMR spectroscopy and mass spectra. The biological efficiency of the ligands and their complexes were examined against the growth of bacteria and fungi in vitro to evaluate their antimicrobial potential. The antibacterial and antifungal assay showed significant activity against the various bacterial and fungal organisms when compared with the standard antibiotics tetracycline and nystatin respectively.



Keywords: Imidazo[4,5-f][1,10]phenanthroline, Aromatic/Heterocyclic aldehydes, Ammonium acetate, Antimicrobial activity, Zinc metal complexes

INTRODUCTION

The invention of cisplatin and its clinical application in cancer therapy promoted serious research for new drug candidates based on coordination compounds [1]. Thus design of new metal-based chemotherapeutic agent is a promising research area of inorganic medicinal chemistry [2]. It is well known that medicinal inorganic chemistry is a multidisciplinary field comprises of chemistry, pharmacology, toxicology and biochemistry. The medicinal chemists have focused on design and synthesis of new metal-based molecules with improved biological activity, better selectivity, lower toxicity and multiple role of mechanistic action to overcome the clinical problems of existing drugs in the market due to its side effects. The literature survey demonstrated that the metal complexes are growth inhibitors of microbes and have been extensively studied *in vitro* and *in vivo*.

The researchers are motivated and search for new metallic species with improved biological applications. Among the metal ions, copper, nickel, cobalt and zinc complexes with a variety of ligands have proved to be an excellent candidate [3]. The ability of metals to lose electrons to form positively charged ions allows metals to play their role in biology. Whereas metal ions are electron deficient, most biological molecules such as protein and DNA are electron rich. The attraction of these opposing forces leads to a general tendency for metal ions to bind and interact with biological molecules [4,5].

Zn(II) is one of most important metal cations in biological systems as it plays an essential role in the activity of nearly 300 enzymes that catalyze approximately 50 important cellular biochemical reactions [4]. At high concentrations Zn(II) shows inhibitory action on the growth of bacteria species like, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [6]. In some cases, the interaction of metal ions (i.e., Zn(II)) with bioactive antibacterial organic compounds increases the biological activity of the ligands but in other cases, the interaction of bioactive organic compounds with metals inhibits their activity, e.g. the anti-bacterial activity of cefadroxil is diminished when it binds to Zn(II) complex [7]. The pharmacological activity has also been found to be highly dependent on the nature of the metal ion and the donor sequence of the ligands as different ligands exhibit different biological properties [8].

There is a great deal of interest in the synthesis and characterization of transition metal complexes of heterocyclic compounds, in particular imidazole derivatives. The heterocyclic moiety benzimidazole plays an important role both in chemical and biological contexts, because it is structurally isostere of naturally occurring nucleotides; hence, it has been extensively utilized as a drug scaffold in the medicinal chemistry. The connection between the wide spectrum of biological activities and compounds containing benzimidazole nucleus has been known and well documented in the literature.

Furthermore, numerous imidazole containing compounds have continued to attract attention for their chemotherapeutic activities [9]. The imidazole ring in organic molecules has been reported to exert significant influence on the intermolecular and intramolecular interactions such as hydrogen bonding, metal–organic coordination strengths, excited state intramolecular proton-electron transfer, etc. [10-13]. Therefore, the electronic nature of the 5-membered imidazole ring could be considered to exert notable influence on the physical properties and chemical reactivity tendencies of imidazole-containing materials.

A lot of research efforts have been concentrated on preparation of transition metal complexes of 1H-imidazo[4,5-f][1,10]phenanthroline (ip) analogues as potential DNA probe molecules. The primary interest has been the search for discovery of DNA probe materials that possess site/pH-specific interactions with certain base residues of known DNA macromolecules [14-17]. With respect to these facts it is quite surprising that literature did not reveal synthesis, characterization and antimicrobial activity to explore the coordination chemistry of transition metal complexes with ligands of heterocyclic substituted 1H-imidazo [4,5-f] [1,10] phenanthroline derivatives. This has prompted us to synthesize and antimicrobial studies of metal complexes involving this ligand. This compound is bidentate macrocyclic ligand having both nitrogens of phenanthroline moiety are the donor atoms. As we know 1,10 phenanthroline has good chelating tendency to form transition metal complex (Figure 1).

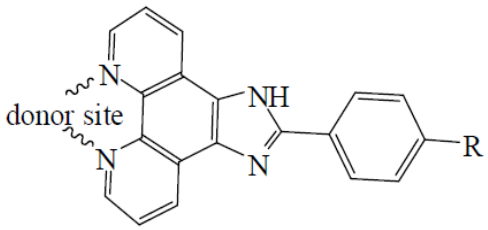


Figure 1: Structure of ligand

MATERIALS AND METHODS

Chemicals, materials and biological species

1,10-phenanthroline, ammonium acetate, zinc chloride were purchased from Alfa Aesar, 4 methyl benzaldehyde, 4-chloro benzaldehyde, indole-3carbaldehyde and 7-azaindole 3-carbaldehyde were purchased from Sigma. All solvents and reagents used were of analytical grade, purchased from local commercial sources and were used as supplied unless otherwise stated. Bacterial and fungal pathogens were procured from the Institute of Microbial Technology (IMTech), Chandigarh, India and National Collection of Industrial Microorganisms (NCIM), Pune, India.

Physical measurements

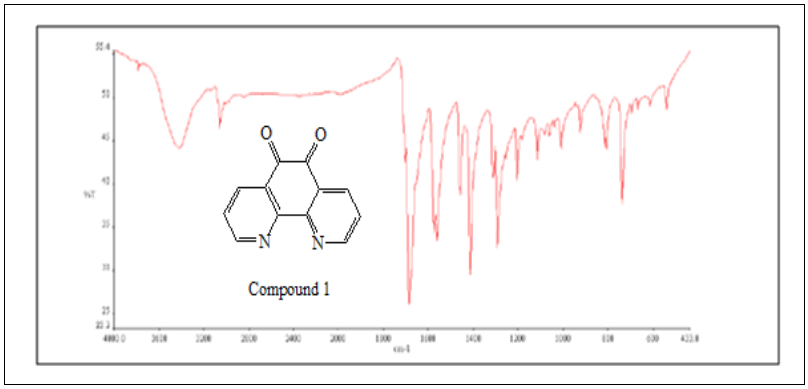
Melting points were recorded in open capillaries with Veego digital melting point apparatus and were uncorrected. Thin layer chromatography (TLC) was performed on silica gel 60 F254 (Merck) precoated aluminium sheets. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a Bruker Avance spectrometer using DMSO-d_6 as solvent and chemical shifts are reported in ppm referred to TMS set at 0.00 ppm. Mass spectra were recorded on a Thermo Scientific LC-MS Orbitrap based system. Elemental microanalysis was performed on Carlo Erba 1108 (CHN) Analyzer.

Synthesis

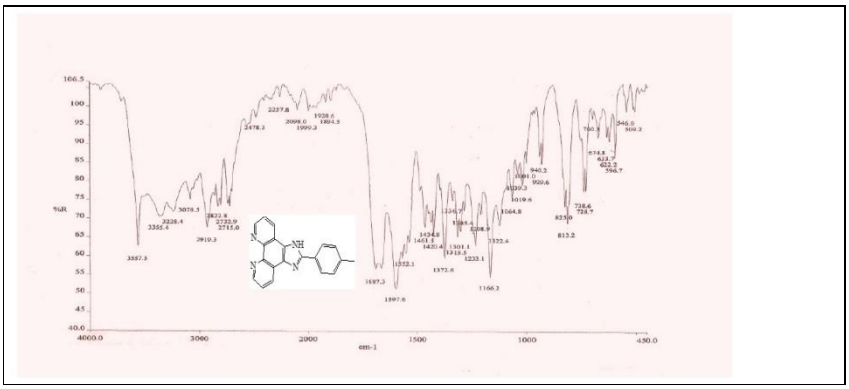
All ligands and their metal complexes were synthesized as shown in Schemes 1 and 2 respectively.

Synthesis of 1,10-phenanthroline-5,6-dione

The synthesis was carried out as per literature process under air 1,10-Phenanthroline (4 g, 22.2 mmol) and KBr (4 g, 33.6 mmol) were thoroughly mixed (by grinding on mortar and pestle) as a solids and slowly (5 min) added to cooled a mixture of H_2SO_4 (98%, 40 ml) and HNO_3 (69%, 20 ml) at temperature 0-5°C. The resulting solution was refluxed (bath temperature 1000C) for 4 h (Caution: The reaction is accompanied by formation of Br_2 fumes). The reaction mixture was cooled to room temperature and poured on to crushed ice (100 g) and cautiously neutralized (Caution: exothermic reaction) with 48% caustic lye solution to pH 4-5 at temperature below 10°C to give a yellow suspension. At higher pH the mixture turns dark green, but addition of acid to pH 4-5 restores the yellow color. The solution was extracted with $CHCl_3$ (2 × 250 ml). Organic phase was washed with water, dried over anhydrous magnesium sulphate, evaporated *in vacuo* and recrystallized from ethanol and dried in vacuum oven.

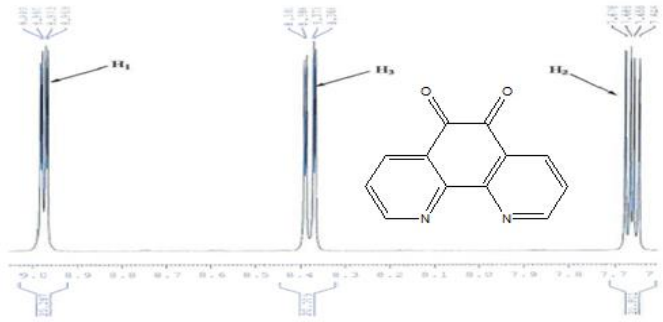


IR Spetrum of 1,10 phenanthroline 5,6 dione

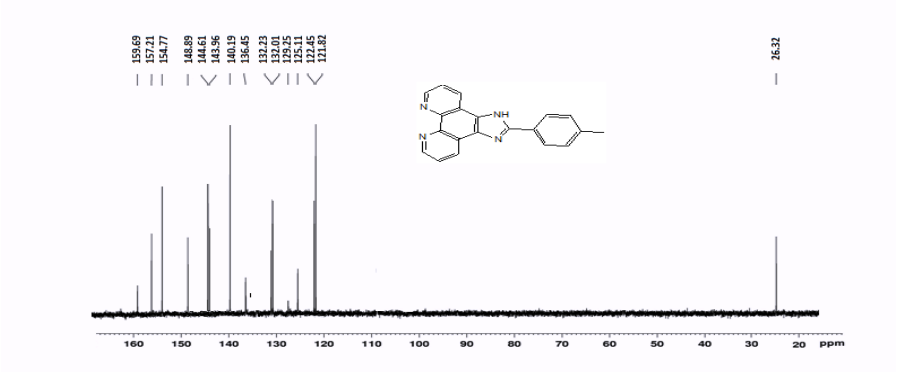


IR Spetrum of compound L[1]

Yield: 85%; MF/FWt: $C_{12}H_6N_2O_2$/210.19; MP: 258-261°C; Elemental composition, Calculated: C, 68.57; H, 2.88; N, 13.30; Found: C, 68.65; H, 2.90; N, 13.24; ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): δ=7.65 (dd, 2H), 8.67 (dd, 2H), 8.97 (dd, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): 125.18, 129.043, 135.617, 152.226, 154.277, 177.688; MS (ESI): m/e 210 (M+).



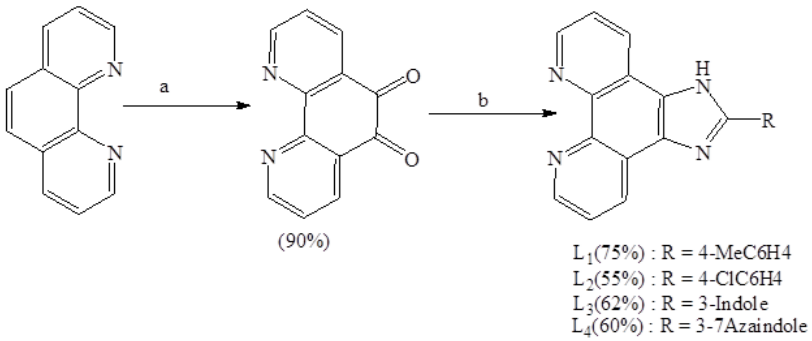
^{1}H-NMR of 1,10 phenanthroline 5,6 dione



^{13}C-NMR of compound 1,10 phenanthroline 5,6 dione

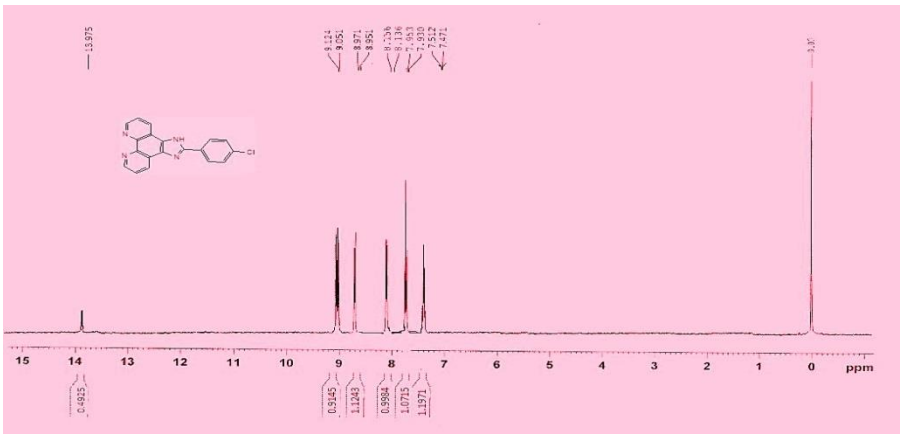
Synthesis of 2-(phenyl or hetero aryl)-1H-imidazo [4,5-f] [1,10] phenanthroline ligands L_1 to L_4: 1,10-Phenanthroline-5,6-dione (10.5 mmol) was dissolved in 100 ml of hot glacial acetic acid to this was added 4-methyl benzaldehyde (10 mmol) L_1/4-chloro benzaldehyde (10 mmol) L_2/Indole-3-aldehyde (10 mmol) L_3 and 7-Azaindole-3-aldehyde (10 mmol) and stirred for 10 min. To this reaction mixture ammonium acetate (67 mmol) was added and stirred at 75-80°C for 5-7 h.

After completion of reaction (TLC), the reaction mixture was allowed to cool to room temperature then poured over crushed ice (25 g) and basified with liquor ammonia. The yellow compound was filtered, washed with water. The obtained product was purified in ethanol, and dried under vacuum.

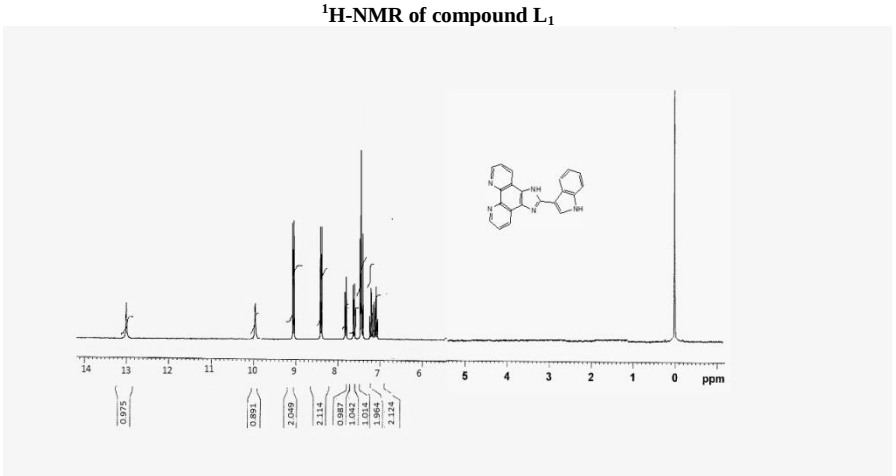


Scheme 1: Synthesis of 1,10-phenonthroline -5,6 dione and Ligand L_1-L_4

Ligand (L_1): Color/Nature: Yellow/solid. Yield: 75%; MF/FWt: $C_{20}H_{14}N_4$/310.35; MP: >300°C; Elemental composition, Calculated: C, 77.40; H, 4.55; N, 18.05. Found: C, 77.21; H,4.51; N, 18.14; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): δ=13.57 (s, 1H, NH), 9.1 (dd, 2H, H-phen), 8.96 (dd, 2H, H-phen), 8.16 (d, 2H, H-Ar), 7.95 (d, 2H, Ar-H), 7.23 (dd, 2H, H-phen), 2.32 (s, 3H, -CH3). ^{13}C-NMR (75 MHz, DMSO-d6, δ ppm): δ 26.32, 121.82, 122.45, 125.11, 129.25, 132.01, 132.23, 136.45, 140.19, 143.96, 144.61, 148.89, 154.77; 157.21; 159.69; MS (ESI): m/e 310 (M+).

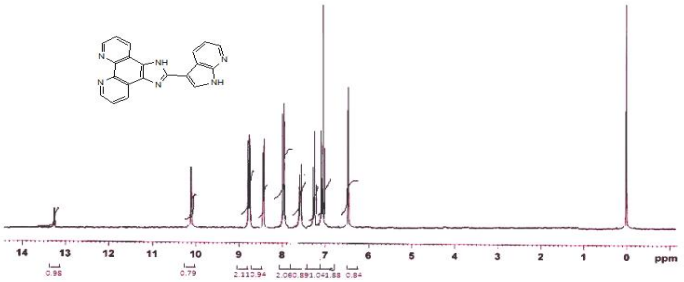


^{1}H-NMR of compound L_1

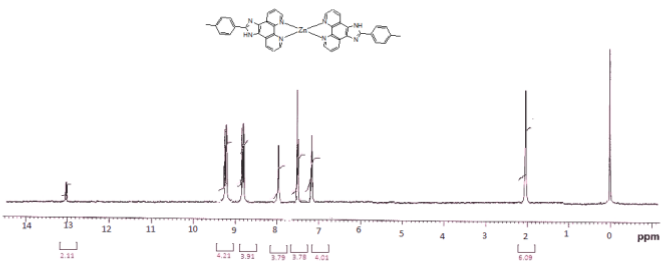


^{13}C-NMR of compound L_1

Ligand (L_2): Color/Nature: Bright yellow/solid. Yield: 55%; MF/FWt: $C_{19}H_{11}ClN_4$/330.5; MP: >300°C; Elemental composition, Calculated: C, 68.99; H, 3.33; N, 16.94. Found: C, 68.94; H, 3.41; N, 17.14; ^{1}H-NMR (300 MHz, DMSO-d6, δ ppm): δ=13.97 (s, 1H, NH), 9.10 (dd, 2H, H-phen), 8.95 (dd, 2H, H-phen), 7.95 (d, 2H, H-Ar), 7.95 (d, 2H, H-Ar), 7.45 (d, 2H, H-phen). ^{13}C-NMR (75 MHz, DMSO-d_6, δ ppm): δ=122.7, 124.6, 126.8, 127.6, 129.0, 129.8, 131.4, 131.9, 139.1, 139.7, 142.6, 151.9, 161.9, 162.7; MS (ESI): m/e 330 (M+).

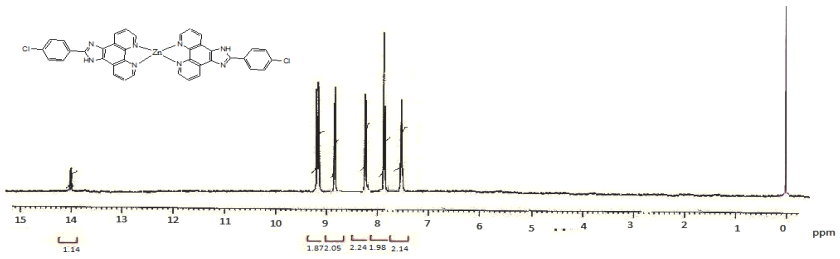


^{1}H-NMR of compound L_2

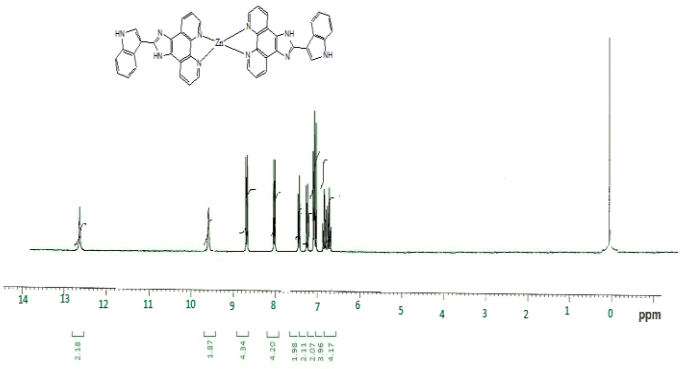


^{13}C NMR of compound L_2

Ligand (L_3): Color/Nature: Bright yellow/solid. Yield: 62%; MF/FWt: $C_{21}H_{13}N_5$/335.35; MP: >300°C; Elemental composition, Calculated: C, 75.21; H, 3.98; N, 20.90. Found: C, 75.75; H, 4.01; N, 19.95; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): δ=13.1 (s, 1H, NH-imidazole), 9.91 (s, 1H, NH-indole), 9.1 (dd, 2H,H-phen), 8.5 (dd, 2H, H-phen), 7.8 (m,1H, H-Ar), 7.7 (m, 1H, H-Ar), 7.5 (m, 1H, H-Pyrrole), 7.3 (dd, 2H, H-phen), 7.2 (m, 1H, H-Ar), 7.1 (m, 1H, H-Ar); ^{13}C-NMR (75 MHz, DMSO-d_6, δ ppm): δ=111.8, 115.1, 119.6, 120.3, 121.8, 125.9, 127.2, 129.0, 130.1, 131.7, 133.2, 137.9, 138.4, 139.5, 142.3, 157.3, 158.1, 159.9; MS (ESI): m/e 335 (M+)

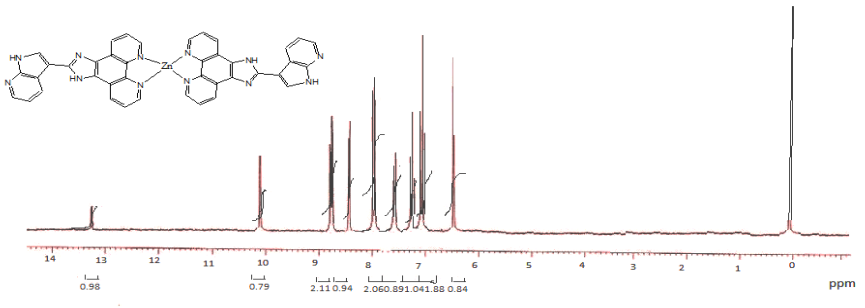


^{1}H-NMR of compound L_3

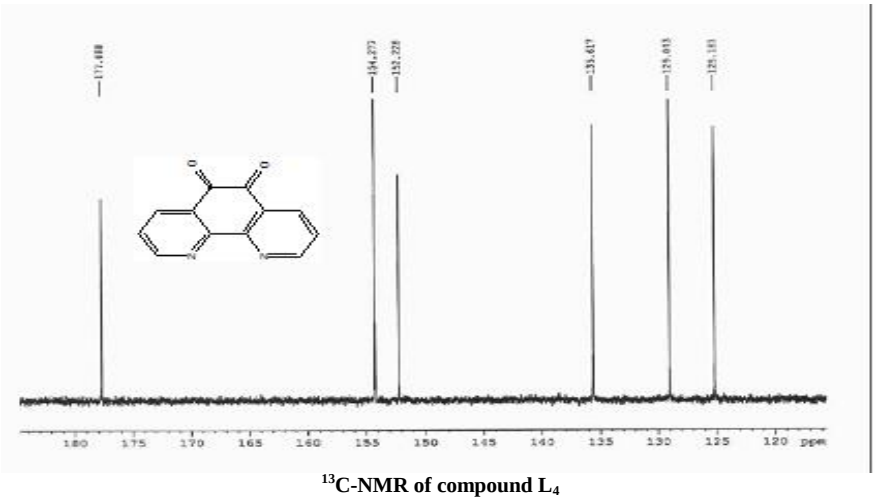


^{13}C-NMR of compound L_3

Ligand (L_4): Color/Nature: Bright yellow/solid. Yield: 60%; MF/FWt: $C_{20}H_{12}N_6$/336; MP: >300°C; Elemental composition, Calculated: C, 71.43; H, 3.57; N, 25.00; Found: C, 71.68; H, 3.47; N, 24.87; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 13.3 (s, 1H, NH-imidazole), 10.1 (s, 1H, NH-indole), 8.9 (dd, 2H, H-phen), 8.5 (m, 1H, H-Ar), 8.0 (dd, 2H, H-phen), 7.8 (m, 1H, H-Ar), 7.4 (m, 1H, H-Ar), 7.1 (dd, 2H, H-phen), 6.5 (s, 1H, H-Pyrrole). ^{13}C-NMR (75 MHz, DMSO-d_6, δ ppm): 101.8, 115.1, 120.3, 121.8, 123.8, 126.0, 127.2, 129.0, 130.1, 131.7, 133.2, 137.4, 142.3, 146.8, 151.7, 157.3, 158.1; MS (ESI): m/e 336 (M+).



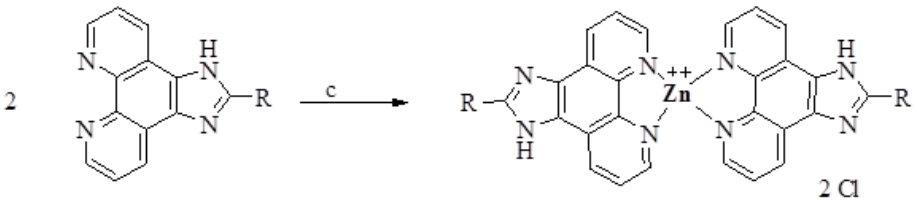
^{1}H-NMR of compound L_4



^{13}C-NMR of compound L_4

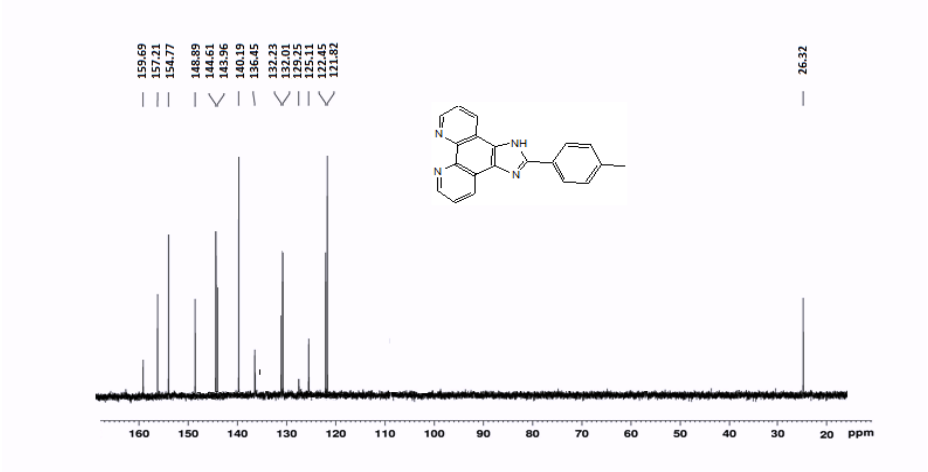
Synthesis of zinc complexes M_1-M_4 from ligands L_1-L_4

The new zinc complexes were prepared according to reported process [18], to an appropriate imidazo[4,5-f]-1,10-phenanthroline derivatives L_1-L_4 (0.54 mmol) dissolved in 10-20 ml of THF-MeOH (1:1, v/v) was added a solution of $ZnCl_2$ (0.27 mmol) in MeOH. A yellow precipitate was formed immediately after addition of the metal salt. The mixture was stirred at room temperature for 24 h and filtered. The solid residue was washed with ethanol and dried in vacuum.



M^1(35%): R=4-MeC6H4
M^2(47%): R=4-Cl C6H4
M^3(42%): R=3 indole
M^4(25%): R=3-7 azaindole

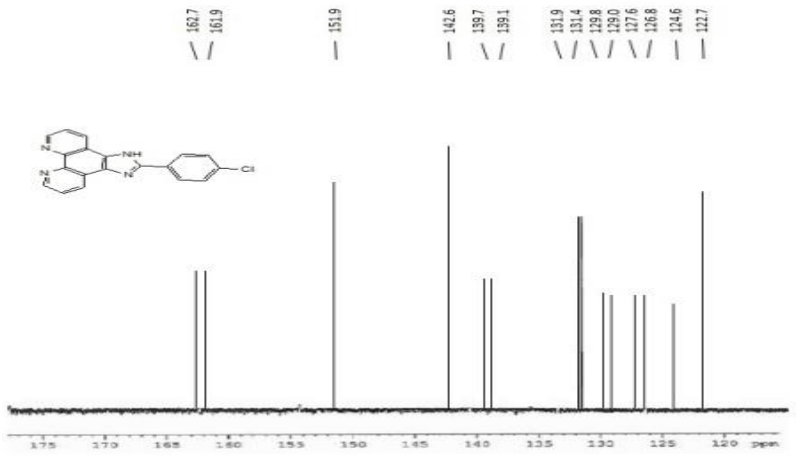
Scheme 2: Synthesis of zinc complexes M_1-M_4



Mass of compound 1,10 phenanthroline 5,6 dione

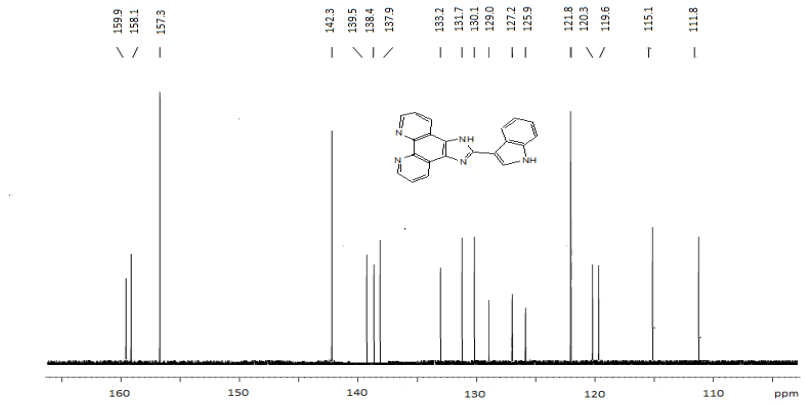
The spectral data of synthesized zinc (II) complexes are as follows,

Zinc complex (M_1): Color/Nature: Yellow/solid; Yield-35%; MF/FWt: $C_{40}H_{28}N_8Zn$/686; MP: >300°C; Elemental composition, Calculated: C, 70.03; H, 4.09; N, 16.34. Found: C, 69.87; H, 3.81; N, 16.21; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): δ=13.1 (s, 2H, 2 × NH), 9.3 (dd, 4H, 2 × H-phen), 8.9 (dd, 4H, 2 × H-phen), 8.0 (m, 4H, 2 × H-Ar), 7.5 ((dd, 4H, 2 × H-phen), 7.2 (m, 4H, 2 × Ar-H), 2.0 (s, 6H, 2 × CH_3).

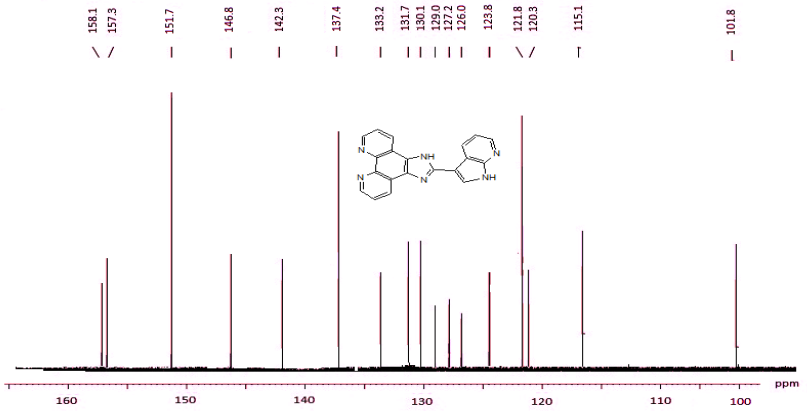


^{1}H-NMR of compound M_1

Zinc complex (M_2): Color/Nature: Yellow/solid; Yield-47%; MF/FWt: $C_{38}H_{22}Cl_2N_8Zn$/726; MP: >300°C; Elemental composition, Calculated: C, 62.78; H, 3.03; N, 15.42. Found: C, 62.00; H, 2.89; N, 15.55; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): δ=14 (s, 2H, 2 × NH), 9.3 (dd, 4H, 2 × H-phen), 8.9 (dd, 4H, 2 × H-phen), 8.2 (m, 4H, 2 × H-Ar), 7.9 (m, 4H, 2 × H-Ar), 7.6 (dd, 4H, 2 × H-phen).

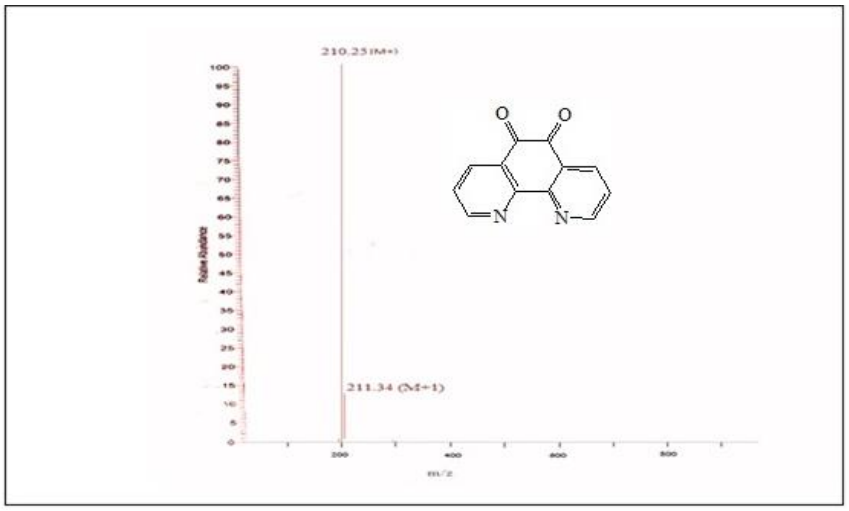


^{1}H NMR of compound M_2

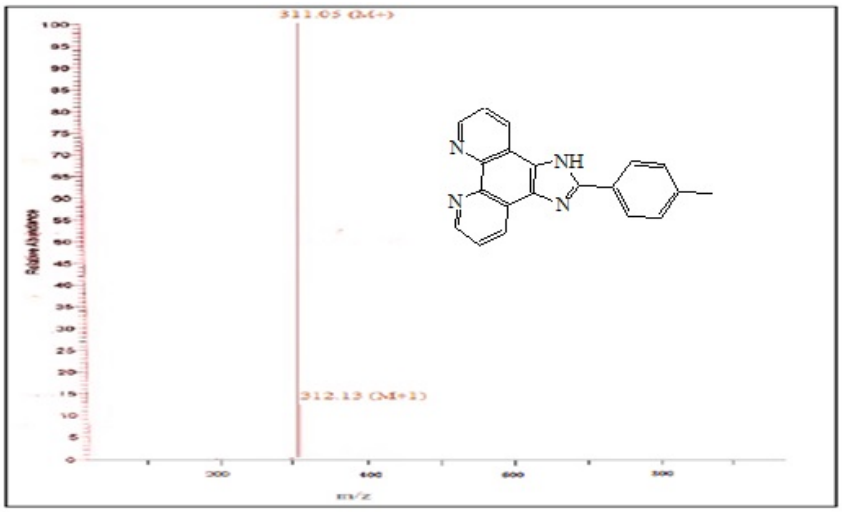


Mass of compound L_2

Zinc complex (M_3): Color/Nature: Pale yellow/solid; Yield-42%; MF/FWt: $C_{42}H_{26}N_{10}Zn$/735; MP: >300°C; Elemental composition, Calculated: C, 68.54; H, 3.54; N, 19.04. Found: C, 69.07; H, 3.61; N, 19.21; 1H NMR (300 MHz, DMSO-d_6, δ ppm): δ=12.8 (s, 2H, 2 × NH imidazole), 9.8 (s, 2H, 2 × NH indole), 8.8 (dd, 4H, 2 × H-phen), 8.0 (dd, 4H, 2 × H-phen), 7.5 (m, 2H, 2 × H-Ar), 7.2 (m, 2H, 2 × H-Ar), 7.1 (m, 2H, 2 × H-Pyrrole), 7.1 (dd, 4H, 2 × H-phen), 6.8 (m, 2H, 2 × H-Ar), 6.6 (m, 2H, 2 × H-Ar).

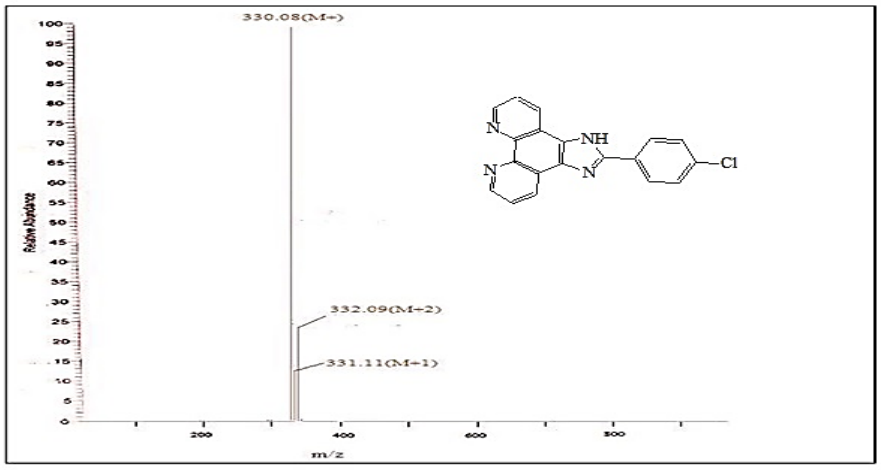


^{1}H-NMR of compound M_3

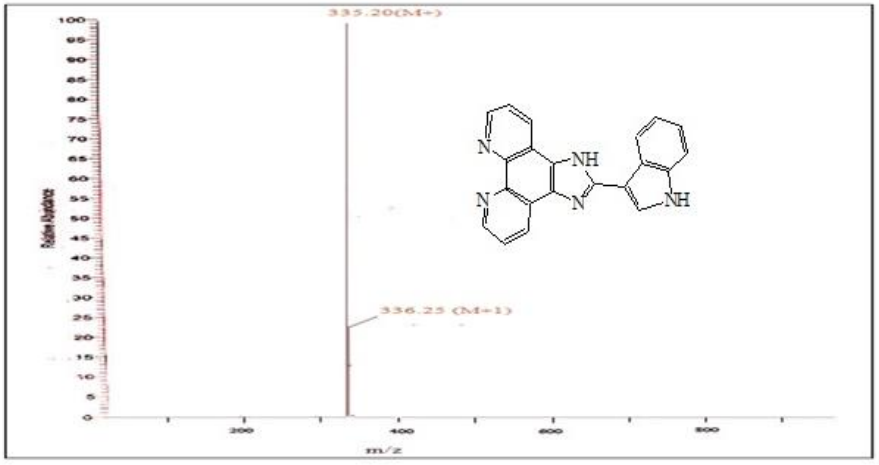


Mass of compound L_3

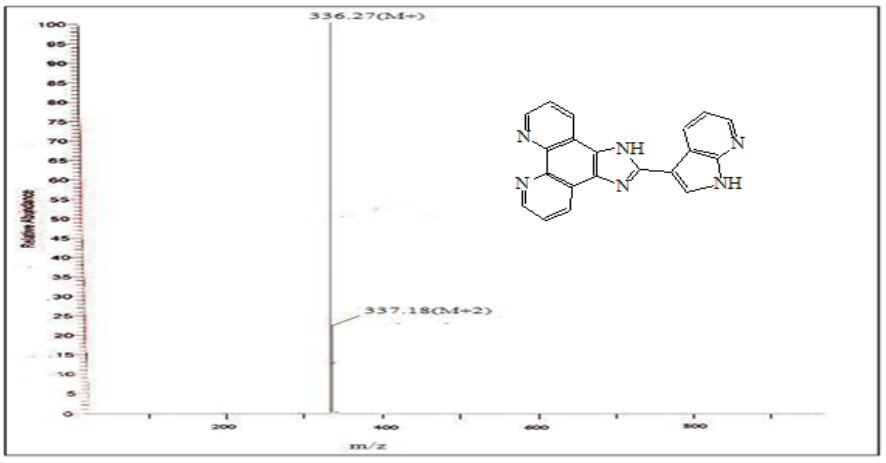
Zinc complex (M_4): Color/Nature: Pale yellow/solid; Yield-25%; MF/FWt: $C_{40}H_{24}N_{12}Zn$/737; MP: >300°C; Elemental composition, Calculated: C, 65.09; H, 3.25; N, 22.78. Found: C, 64.94; H, 3.11; N, 23.01;. ^{1H}-NMR (300 MHz, DMSO-d_6, δ ppm): 13.4 (s, 2H, 2 × NH-imidazole), 10.1 (s, 2H, 2 × NH-indole), 8.9 (dd, 4H, 2 × H-phen), 8.5 (m, 2H, H-pyridyl), 8.0 (dd, 4H, 2 × H-phen), 7.6 (m, 2H, 2 × H-pyridyl), 7.4 (m, 2H, 2 × H-pyridyl), 7.1 (dd, 4H, 2 × H-phen), 6.5 (s, 2H, 2 × H-Pyrrole).



^{1}H-NMR of compound M_4



Mass of compound L^3



Mass of compound L^4

Biological evaluation

Antimicrobial activity of all synthesized compounds was determined by agar diffusion method [19,20]. All human pathogenic bacterial strains viz. *Escherichia coli* (MTCC 1650), *Proteus vulgaris* (MTCC 1771), *Klebsiella pneumoniae* (NCIM 2957), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 1789), *Aspergillus niger* (MTCC 1781) and fungal strains viz. *Trichoderma viridae* (MTCC 167), *Aspergillus flavus* (MTCC 2501) were used for antimicrobial study of newly synthesized ligands L_1-L_4 and zinc complexes M_1-M_4.

All the synthesized compounds were dissolved to prepare a stock solution of 1 mg/ml using Dimethyl Sulfoxide (DMSO) (0.05%). Stock solution was aseptically transferred and suitably diluted to have solutions of concentration ranging 50-100 µg. For antifungal activity, different fungal spore suspensions in sterile distilled water were adjusted to give a final concentration of 106 cfu/ml. Inoculums of 0.1 ml spore suspension of each fungus were spread on Sabouraud's Dextrose agar plates (HiMedia). For antibacterial activity Muller Hinton agar was used (HiMedia) seeded with 0.1 ml of the respective bacterial culture strains suspension prepared in a sterile saline (0.85%) of 105 cfu/ml dilution. The wells of 6 mm diameter were filled with 0.1 ml each test compound separately for fungus and bacterial strain. The DMSO (0.05%) alone was used as a controller.

The antibiotics tetracycline and nystatin were used as a reference for antibacterial and antifungal, respectively. Inoculated plates in duplicate were then incubated for 24 h at 37 ± 0.5°C for antibacterial activity and 48 h at 28 ± 0.2°C for antifungal activity. After incubation the antimicrobial activity was measured in terms of the zone of inhibition in mm.

RESULTS AND DISCUSSION

Synthesis and characterization

The 1H-imidazo [4,5-f] [1,10] phenanthroline heterocyclic bidentate ligands L_1-L_4 were synthesized using a simple two-step approach starting from commercially available 1,10-phenanthroline. 1,10-Phenanthroline-5, 6-dione readily reacted with various aldehydes to give compounds L_1-L_4 in up to 75% yield (Scheme 1). The metal complexes M_1-M_4 were prepared using the ligands L_1-L_4 and the metal salt in good yields. Zn(II) complexes were synthesized using $ZnCl_2$ in THF-MeOH in fair yields up to 47% via a standard approach in which the metal ions and the hetero atom from ligand coordinate each other (Scheme 2).

The analytical data of the ligands and their metal (II) complex were summarized in the experimental section. They are well agreed with the theoretical values within the limit of experimental error. ^{1}H-imidazo [4,5-f] [1,10] phenanthroline ligands (L_1-L_4) and their Zn(II) complexes (M_1-M_4) possesses intense yellow colors. Newly synthesized metal complexes are quite stable in air and light. Decomposition points of the complexes are relatively high as these are stable up to temperature >300°C suggesting good thermal stability [15]. They have very good solubility in Dimethyl Fluoride (DMF) and DMSO, but were hardly soluble in common solvents, such as CH_2Cl_2 and $CHCl_3$. The poor solubility of metal complexes fails the attempts to prepare good quality crystals. The structure of ligands and their metal complexes were established using elemental analyses, ^{1}H-NMR, ^{13}C-NMR and Mass spectra. Physical data of the Zn(II) metal complexes and ^{1}H-imidazo [4,5-f] [1,10] phenanthroline ligands are summarized in the Table 1.

Table 1: Physical data of synthesized compounds

Compound	Color	Nature	M.P. °C	% Yield
1,10 phen 5,6 dione	Yellow	Solid	260	85
L^1	Yellow	Solid	>300	75
L^2	Bright Yellow	Solid	>300	55
L^3	Bright Yellow	Solid	>300	62
L^4	Bright Yellow	Solid	>300	60
M^1	Yellow	Solid	>300	35
M^2	Yellow	Solid	>300	47
M^3	Pale Yellow	Solid	>300	42
M^4	Pale Yellow	Solid	>300	25

Elemental analysis

All the newly synthesized ligands and complexes were subjected to elemental analysis for determining the percentage compositions of each element as well as metal to ligand ratio. The observed and calculated values were summarized in Table 2. From elemental analysis, the complex is of ML_2 type that is one central metal formed complex with two ligands.

Table 2: Elemental analysis (%C, %H, %N)

Compound	Molecular formula	Molecular weight g/mol	% Elemental analysis					
			% C		% H		% N	
			Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
1,10-phen-5,6-dione	$C_{12}H_6N_2O_2$	210.19	68.57	68.65	2.86	2.9	13.33	13.24
L^1	$C_{20}H_{14}N_4$	310.35	77.42	77.21	4.52	4.51	18.06	18.14
L^2	$C_{19}H_{11}ClN_4$	330.5	68.99	68.94	3.33	3.41	16.94	17.14
L^3	$C_{21}H_{13}N_5$	335	75.22	75.75	3.88	4.01	20.9	19.95
L^4	$C_{20}H_{12}N_6$	336	71.43	71.68	3.57	3.47	25	24.87
M^1	$C_{40}H_{28}N_8Zn$	686	70.03	69.87	4.09	3.81	16.34	16.21
M^2	$C_{38}H_{22}Cl_2N_8Zn$	726	62.78	62	3.03	2.89	15.42	15.55
M^3	$C_{42}H_{26}N_{10}Zn$	735	68.54	69.07	3.54	3.61	19.04	19.21
M^4	$C_{40}H_{24}N_{12}Zn$	737	65.09	64.94	3.25	3.11	22.78	23.01

^{1}H-NMR spectra

The structures of ligands and their zinc complexes were recorded in DMSO as solvent and Tetramethylsilane (TMS) as internal standard. The chemical shifts (δ) of characteristic protons in these compounds are expressed in ppm and summarized in the experimental section. In ^{1}H-NMR spectra of ligands (L_1-L_4) one single –NH proton of imidazole ring appeared as singlet and observed in the δ=12-14 ppm regions, each integrating for one proton. ^{1}H-NMR spectra of zinc complexes also shows singlet in the δ=12-13.5 ppm regions means imidazole ring proton is present and nitrogen of imidazole ring not involved in the complex formation. The characteristic aromatic and phenanthroline protons of ligands were appeared in aromatic region δ=7-9 ppm while that of complexes also appears in aromatic region but slight distortions in δ values indicating 1,10 phenanthroline nucleus involving complex formation. Further the methyl -CH_3 protons in L_1 and M_1 will appear at the range of δ=2-2.5 ppm. Also the NH protons of indole and azaindole nucleus of L_3, M_3 and L_4, M_4 respectively will appear at around δ=9.8-10.2 ppm suggesting that indole nitrogens were not involved in the complex formation. All compounds chemical shift value of were summarized in Table 3.

Table 3: ^{1}H-NMR signal in δ ppm

Compound	methyl protons	phenanthroline ring protons	Imidazole (-NH) protons	phenyl ring protons	Indole/Azaindole ring protons
1,10-phen-5,6-dione	-	7.65, 8.67, 8.97	-	-	-
L^1	2.32	9.1, 8.96, 7.23	13.57,	8.16, 7.95	-
L^2	-	9.10, 8.95, 7.45	13.97	8.05, 7.95	-
L^3	-	9.1, 8.5, 7.3	13.1	-	9.91, 7.8, 7.7, 7.5, 7.2, 7.1.
L^4	-	8.9, 8.0, 7.1	13.3	-	10.1, 8.5, 7.8, 7.4, 6.5
M^1	2.00	9.3, 8.9, 7.5	13.1	8.0, 7.2	-
M^2	-	9.3, 8.9, 7.6	14	8.2, 7.9	-
M^3	-	8.8, 8.0, 7.1	12.8	-	9.8, 7.5, 7.2, 7.1, 6.8, 6.6
M^4	-	8.9, 8.0, 7.1	13.4	-	10.1, 8.5, 7.6, 7.4, 6.5

^{13}C-NMR spectra

The ^{13}C-NMR spectrum of the 1,10-phen-5,6-dione, total six signals appeared as there is presence of six set of carbons. Since all carbons are of hetero aromatic ring, so they appear at δ=125.18, 129.043, 135.617, 152.226, 154.277 and 177.688. The signal at δ=177.688 ppm is due to the carbonyl carbon, while the peak at δ=154.277 is for the 2 ad 9 positions carbon, appeared downfield because of neighboring electron donating nitrogen atom. Remaining signals appearing at aromatic region.

In compounds L_1-L_4, there is no signal at around δ=175 ppm means absence of carbonyl carbon. Signals at around δ=157 ppm is characteristic peak of carbon situated between two nitrogens of imidazole ring. In compound L_4 the signal at δ=26.3 ppm is due to methyl carbon while remaining signals are much more similar to 1,10-phen-5,6-dione and imidazole ring. Further, in complexes M_1-M_4 the signals were similar to L_1-L_4 respectively but there is slight downfield chemical shift were observed which may be attributed to metal coordination.

Biological screening

Antimicrobial activity (agar diffusion method)

All the newly synthesized ^{1}H-imidazo [4,5-f] [1,10] phenanthroline ligands and their Zn(II) complexes were evaluated for their *in vitro* anti-microbial activity. In this study, 9 newly synthesized phenanthroline derivatives were subjected to antimicrobial study by *in vitro* disk diffusion method against bacterial strains viz. *Escherichia coli* (MTCC 1650); *Proteus vulgaris* (MTCC 1771); *Klebsiella pneumoniae* (NCIM 2957); *Staphylococcus aureus* (MTCC 96); *Bacillus subtilis* (MTCC 1789) and fungal strains viz. *Aspergillus niger* (MTCC 1781); *Trichoderma viridae* (MTCC 167); *Aspergillus flavus* (MTCC 2501). The diameter of the zone of inhibition (mm) was used to compare the antimicrobial activity of the test compound with the commercial drugs. Nystatin and tetracycline are used as standard drugs against fungi and bacteria, respectively (Figures 2a and 2b). The results are reported as a mean of duplicate measurements.

The results of antimicrobial activity of novel ligands and their metal complexes are found to be comparable with standard drugs, presented in Table 4. Results show that almost all the synthesized compounds possess good antibacterial activity. It is observed that out of the 9 compounds screened against three Gram negative strains *E. coli*, *P. vulgaris*, *K. pneumoniae* and two Gram positive strains *S. aureus*, *B. subtilis*, the compounds L_3, L_4, M_1-M_4 are active against both Gram positive and Gram negative strains, whereas as some compounds viz. 1,10-phen 5,6-dione, L_1 and L_2 are inactive against selected bacterial strains. Compounds M_1-M_4 has shown excellent antibacterial activity as compared to that of standard drug at tested concentration. Interestingly, compounds L_1-L_4 showing moderate antibacterial activity. The difference in activities is due to the presence of zinc metal in M_1-M_4. In addition, it was observed that heterocyclic ring substituent on phenanthroline ring showed higher antibacterial activity. Phenanthroline mimics containing cyclic aromatic moieties as substitutents have shown moderate antibacterial activity.

Compounds L_2, L_3, L_4, M_1, M_2, M_3 and M_4 showed strong antifungal activity as compared to standard drug nystatin at tested concentration. Some of the compounds are inactive against selected fungal strains. Structure activity relationship study indicates that compounds containing complexation of ligand with zinc metal have shown higher antifungal activity than their ligands. Phenanthroline derivatives containing heterocyclic ring substituent on phenanthroline ring showed higher antifungal activity than their counterparts of cyclic aromatic substituent.

Table 4: Antimicrobial results

Compound	Bacteria (MIC at 50 µg/ml)					Fungi (MIC at 100 µg/ml)		
	EC	KN	PV	SA	BS	AN	TV	AF
1,10-phen 5,6-dione	**5**	**ND**	**ND**	**8**	**9**	**7**	**ND**	**11**
L^1	7	10	ND	6	10	ND	11	9
L^2	8	ND	7	6	ND	11	4	8
L^3	9	8	11	9	8	10	14	10
L^4	8	11	10	12	9	8	11	10
M^1	12	13	6	10	11	8	11	8
M^2	12	13	6	10	11	9	15	10
M^3	10	18	15	13	14	14	12	12
M^4	14	15	12	20	12	12	14	15
Tetracycline	15	16	20	18	18	ND	ND	ND
Nystatin	ND	ND	ND	ND	ND	11	12	11

Comparative study of zone of inhibition for bacterial and fungal growth with standard antibiotics

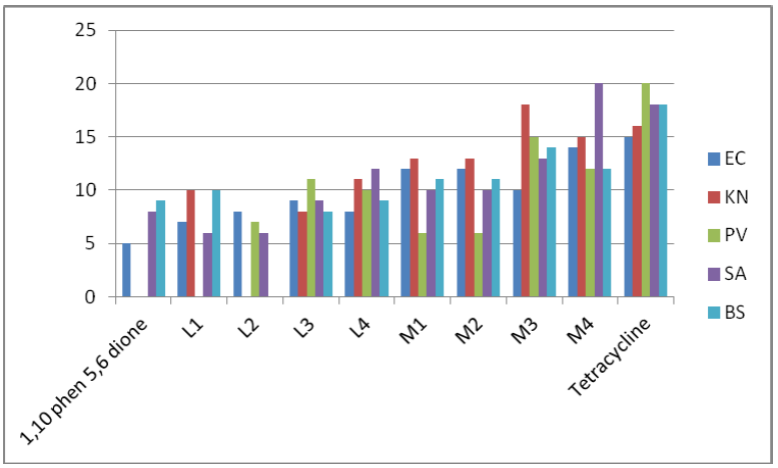


Figure 2a: Comparative study of inhibition of bacterial growth

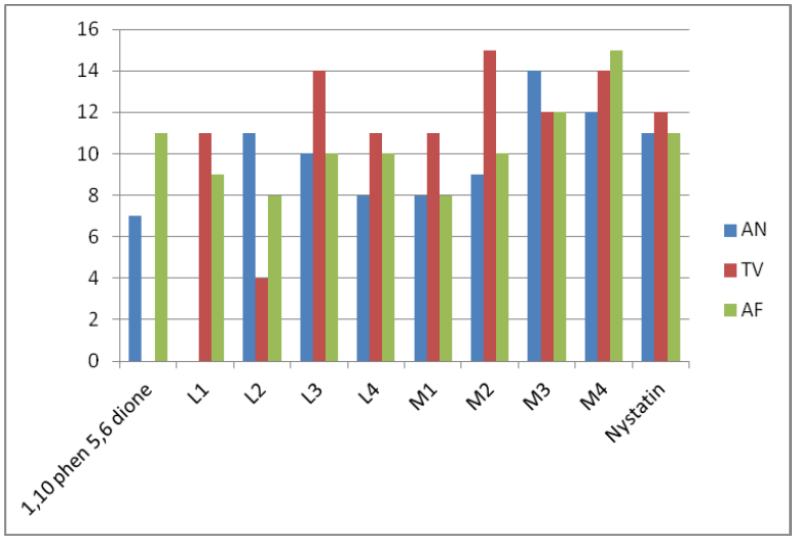


Figure 2b: Comparative study of inhibition of fungal growth

CONCLUSION

Present investigation shows that the bidentate ligand derived by multicomponent reaction of 1,10-phenanthroline-5,6-dione, aromatic/heterocyclic aldehyde and ammonium acetate and 1,10-phenanthroline moiety coordinates readily with metal salts afford the synthesis of complexes. However detailed spectroscopic study including elemental analysis, NMR and mass was needed to investigate the influence of structure and coordination on the reactivity of the corresponding ligand. Elemental analysis and NMR spectral study confirms the metal to ligand ratio is 1:2. Also thermal studies have shown a good thermal stability of complexes. An investigation of biological behavior of all synthesized species has shown significant antibacterial and antifungal results. A detailed structural and biological investigation of this series of complexes would throw more light on the influence of metal coordination on the reactivity of macrocyclic molecules which may be further explored and used as alternative therapeutic agents.

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REFERENCES

[1] R.B. Weiss, M.C. Christian, *Drugs.*, **1993**, 46, 360-377.
[2] S. Sathiyaraj, K. Sampath, R.J. Butcher, R. Pallepogu, C. Jayabalakrishnan, *Eur. J. Med. Chem*., **2013**, 64, 81-89.
[3] E. Wong, C.M. Giandomenico, *Chem. Rev*., **1999**, 99, 2451-2466.
[4] J.J.R.F. da Silva, R.J.P. Williams, Clarendon Press, Oxford, **1991**.
[5] R. Uaucu, N.G. Karaburun, I. Isikdag, *Farmaco*., **2001**, 56, 285-290.
[6] M.S. Khan, S.A. Siddiqui, M.S.R.A. Siddiqui, U. Goswami, K.V. Srinivasan, M.I. Khan, *Chem. Biol. Drug Des*., **2008**, 72, 197-204.
[7] M. McCann, B. Coyle, J. Briody, F. Bass, N. O'Gorman, M. Devereux, K. Kavanagh, V. McKee, *Polyhedron*., **2003**, 22, 1595-1601.

[8] B.K. Keppler, VCH, Weinheim, **1993**.
[9] Y. Sato, Y. Onozaki, T. Sugimoto, H. Kurihara, K. Kamijo, C. Kadowaki, T. Tsujino, A. Watanabe, S. Otsuki, M. Mitsuya, M. Iida, K. Haze, T. Machida, Y. Nakatsuru, H. Komatani, H. Kotani, Y. Iwasawa, *Bioorg. Med. Chem. Lett*., **2009**, 19, 4673-4678.
[10] J. Joseph, M. Sirajul Muneera, *Der Pharma Chemica.*, **2016**, 8, 45-60.
[11]T.F. Markle, I.J. Rhile, A.G. DiPasquale, J.M. Mayer, *Proc. Natl. Acad. Sci. U.S.A*., **2008**, 105, 8185-8190.
[12] Y.P. Tong, S.L. Zheng, X.M. Chen, *Eur. J. Inorg. Chem*., **2005**, 18, 3734-3741.
[13] I.J. Rhile, T.F. Markle, H. Nagao, A.G. Di Pasquale, O.P. Lam, M.A. Lockwood, K. Rotter, J.M. Mayer, *J. Am. Chem. Soc*., **2006**, 128, 6075-6088.
[14] H. Xu, K.C. Zheng, Y. Chen, Y.Z. Li, L.J. Lin, H. Li, P.X. Zhang, L.N. Ji, *Dalton Trans*., **2003**, 11 2260-2268.
[15] P. Nagababu, S. Satyanarayana, *Polyhedron.*, **2007**, 26, 1686-1692.
[16] Y.J. Liu, X.Y. Guan, X.Y. Wei, L.X. He, W.J. Mei, J.H. Yao, *Transition Met. Chem*., **2008**, 33, 289-294.
[17] A.S. Denisova, M.B. Degtyareva, E.M. Dem'yanchuk, A.A. Simanova, *Russ. J. Organ. Chem*., **2005**, 41, 1690-1693.
[18] N. Sergeeva, M. Marechal, G. Vaz, A. Davis, M. Senge, *J. Inorg. Biochem*., **2011**, 105, 1589-1595.
[19] R.W. Fairbrother, G. Martyn, *J. Clin. Pathol*., **1951**, 4, 374.
[20] J.C. Gould, J.H. Bowie, *J. Edinb. Med*., **1952**, 59, 178.