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ANTIMICROBIAL AND SPECTRAL STUDIES OF ZINC METAL COMPLEXES DERIVED FROM IMIDAZO [4, 5-F] [1, 10] – PHENANTHROLINE DERIVATIVE

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ABSTRACT

The paper presents the synthesis of new zinc metal complexes derived from imidazo [4, 5- f] [1, 10] phenanthroline derivatives. All newly synthesized ligands & complexes were characterized by combination of elemental analysis, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. Also, all the newly synthesized imidazo [4, 5-f] [1, 10] phenanthroline ligands and their zinc complexes were evaluated for their *in vitro* anti-microbial activity against the growth of bacteria and fungi to evaluate their antimicrobial potential. These studies revealed that the new complexes were found to have significant activity against the various bacterial and fungal organisms when compared with the standard antibiotics tetracycline and nystatin respectively and be more active than the parent ligand.

KEYWORDS: Imidazo [4, 5-*f*] [1, 10] phenanthroline, Antibacterial activity, 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 inspired for search 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 affinity for metal ions to bind and interact with biological molecules.[4, 5]

Zinc 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 zinc 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 anti-bacterial 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 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.[14-16] 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.

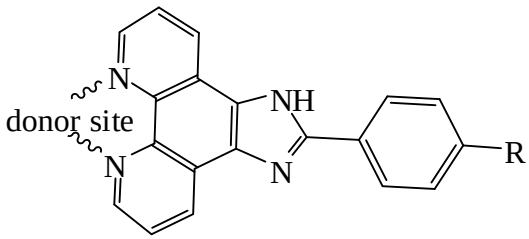


Figure 1: Structure of ligand imidazo [4, 5-f] [1, 10] phenanthroline derivative.

MATERIALS AND METHODS

MATERIALS

1, 10-Phenanthroline, Ammonium Acetate, Zinc Chloride were purchased from Alfa Aesar, 4 Methyl Benzaldehyde, 4-Chloro Benzaldehyde and 7 - Azaindole 3 - Carbaldehyde were purchased from Sigma. Indole - 3 Carbaldehyde were synthesized according to the literature procedure.[17] All solvents and reagents used were of analytical grade, purchased from local commercial sources and were used as supplied. Bacterial and fungal pathogens were procured from the Institute of Microbial Technology (IMTech), Chandigarh, India and National Collection of Industrial Microorganisms (NCIM), Pune, India.

METHODS

Thin layer chromatography (TLC) was performed on silica gel 60 F254 (Merck) precoated aluminum sheets and component spots were detected on UV light at 254 nm. Melting points were recorded in open capillaries with Veego digital melting point apparatus and were uncorrected. ^{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. IR and Mass spectra were recorded on Thermo Scientific Nicolet iS50 FT-IR Spectrometer and on a Thermo Scientific LCMS Orbitrap based system respectively. Elemental microanalysis was performed on Carlo Erba 1108 (CHN) Analyzer.

SYNTHESIS

1. Synthesis of 1, 10-phenanthroline 5, 6 Dione (1)

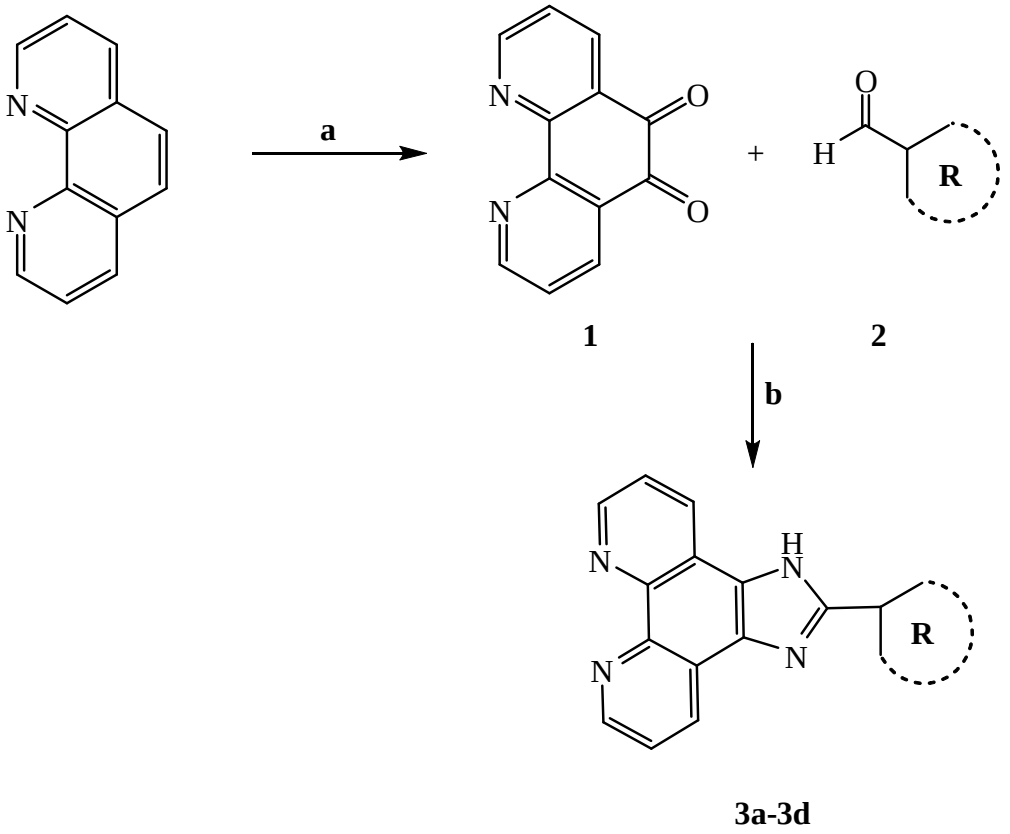
The synthesis was carried out as per literature process[18] under air. 1, 10-Phenanthroline (4 g, 22.2 mmol) and KBr (4 g, 33.6 mmol) were thoroughly mixed (by grinding) as a solids and slowly (5 min) added to cooled a mixture of H_2SO_4 (98%, 40 ml) and HNO_3 (69%, 20 ml) at temperature 0-5 $^{\circ}$C. The resulting solution was refluxed (bath temperature 100^{0}C) for 4 hr (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 (100g) 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 $CHCl_3$ (2x250 ml). Organic phase was washed with water, dried over anhydrous magnesium sulphate, evaporated in vacuo, and recrystallized from ethanol and dried in vacuum oven.

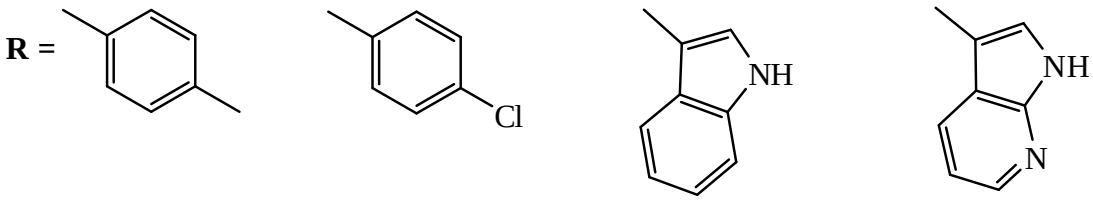
Yield: 85%; MF / FWt: $C_{12}H_6N_2O_2$ / 210.19; MP: 258-261 ^{o}C; Elemental composition, Calculated: C, 68.57; H, 2.88; N, 13.30; Found: C, 68.65; H, 2.90; N, 13.24; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.65 (dd, 2H), δ 8.67 (dd, 2H), δ 8.97 (dd, 2H) ; ^{13}C NMR (75 MHz, $CDCl_3$): δ 125.18, δ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

2. Synthesis of imidazo [4, 5-f] [1, 10] phenanthroline derivatives (3a-3d)

Compound **1** (10.5 mmol) was dissolved in 100 ml of hot glacial acetic acid. To this reaction mixture, aromatic or hetero aromatic aldehydes **2a-2d** (10 mmol) was then added and stirring continued and stirred for 10 min. To this reaction mixture ammonium acetate (67 mmol) was

added and stirred at 75–80 °C for 5-7 hrs. After completion of reaction (TLC), the reaction mixture was allowed to cool to room temperature then poured over crushed ice (25g) and basified with liquor ammonia. The yellow compound was filtered, washed with water. The obtained crude product was purified in ethanol, and dried under vacuum.





(a) **(b)** **(c)** **(d)**

Scheme 1: Reagents and conditions: (a) HNO_3, H_2SO_4, 0°C-100°C, 4h ; (b) NH_4OAc, AcOH, EtOH, 75-80°C, 7h.

The spectral data of synthesized imidazo [4,5-f][1,10]phenanthroline are as follows.

2.1. Synthesis of 2-p-tolyl-1H-imidazo[4,5-f][1,10]phenanthroline (3a)

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), δ 9.1 (dd, 2H, H-phen), δ 8.96 (dd, 2H, H-phen), δ 8.16 (d, 2H, H-Ar), δ 7.95 (d, 2H, Ar-H), δ 7.23 (dd, 2H, H-phen), δ 2.32 (s, 3H, -CH_3); ^{13}C NMR (75 MHz, DMSO-d^6, δ ppm): δ 26.32, δ 121.82, δ 122.42, δ 125.11, δ 129.25, δ 132.23, δ 132.01, δ 136.45, δ 140.19, δ 143.96, δ 144.61, δ 148.89, δ 154.77, δ 157.21, δ 159.69; MS (ESI): m/e 310 (M^+).

2.2. Synthesis of 2-p-chloro-1H-imidazo[4,5-f][1,10]phenanthroline (3b)

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-d^6, δ ppm): δ 13.97 (s, 1H, NH), δ 9.10 (dd, 2H, H-phen), δ 8.95 (dd, 2H, H-phen), δ 7.95 (d, 2H, H-Ar), δ 7.95 (d, 2H, H-Ar), δ 7.45 (d, 2H, H-phen); ^{13}C NMR (75 MHz, DMSO-d^6, δ ppm): δ 122.7, δ 124.6, δ 126.8, δ 127.6, δ 129.0, δ 129.8, δ 131.4, δ 131.9, δ 139.1, δ 139.7, δ 142.6, δ 151.9, δ 161.9, δ 162.7; MS (ESI): m/e 330 (M^+).

2.3. Synthesis of 2-(1H-indol-3-yl)-1H-imidazo[4,5-f][1,10]phenanthroline (3c)

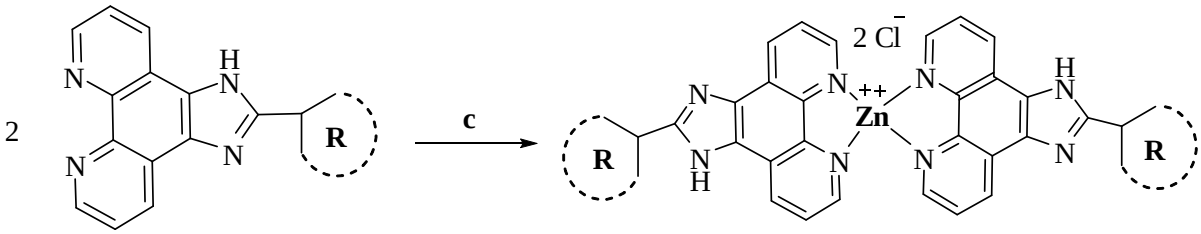
Yield: 62%; MF / FWt: $C_{21}H_{13}N_5$/ 335.35; MP: >300 °C; Elemental composition, Calculated: C, 75.21; H, 3.98; N, 20.90; Found: C, 75.75; H, 4.01; N, 19.95; ^{1}H NMR (300 MHz, DMSO-d^6, δ ppm): δ 13.1 (s, 1H, NH-imidazole), δ 9.91 (s, 1H, NH-indole), δ 9.1 (dd, 2H,H-phen), δ 8.5 (dd, 2H, H-phen), δ 7.8 (m,1H, H-Ar), δ 7.7 (m, 1H, H-Ar), δ 7.5 (m, 1H, H-Pyrrole), δ 7.3 (dd, 2H, H-phen), δ 7.2(m, 1H, H-Ar), δ 7.1(m, 1H, H-Ar); ^{13}C NMR (75 MHz, DMSO-d^6, δ ppm): δ 111.8, δ 115.1, δ 119.6, δ 120.3, δ 121.8, δ 125.9, δ 127.2, δ 129.0, δ 130.1, δ 131.7, δ 133.2, δ 137.9, δ 138.4, δ 139.5, δ 142.3, δ 157.3, δ 158.1, δ 159.9; MS (ESI): m/e 335 (M^+).

2.4. Synthesis of 2-(1H-7-Azaindol-3-yl)-1H-imidazo[4,5-f][1,10]phenanthroline (3d)

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): δ 13.3 (s, 1H, NH-imidazole), δ 10.1 (s, 1H, NH-indole), δ 8.9 (dd, 2H, H-phen), δ 8.5 (m, 1H, H-Ar), δ 8.0 (dd, 2H, H-phen), δ 7.8 (m,1H, H-Ar), δ 7.4 (m, 1H, H-Ar), δ 7.1 (dd, 2H, H-phen), δ 6.5 (s, 1H, H-Pyrrole); ^{13}C NMR (75 MHz, DMSO-d^6, δ ppm): δ 101.8, δ 115.1, δ 120.3, δ 121.8, δ 123.8, δ 126.0, δ 127.2, δ 129.0, δ 130.1, δ 131.7, δ 133.2, δ 137.4, δ 142.3, δ 146.8, δ 151.7, δ 157.3, δ 158.1; MS (ESI): m/e 336 (M^+).

3. Synthesis of zinc complexes of imidazo [4, 5-f] [1, 10] phenanthroline derivatives (4a-4d)

The new zinc complexes were prepared according to reported process [19], to an appropriate imidazo [4, 5-f]-1, 10-phenanthroline derivatives **3a-3d** (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 reaction was monitored by TLC. After completion of reaction, reaction mass was filtered and solid residue was washed with ethanol and dried in vacuum.



3a-3d **4a-4d**

Scheme 2: Reagents and conditions: (c) ZnCl2, THF, MeOH, RT, 24h.

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

3.1. Synthesis of zinc complexes of 2-p-tolyl-1H-imidazo[4,5-f][1,10]phenanthroline (4a)

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, 2xNH), δ 9.3 (dd, 4H, 2xH-phen), δ 8.9 (dd, 4H, 2xH-phen), δ 8.0 (m, 4H, 2xH-Ar), δ 7.5 ((dd, 4H, 2xH-phen), δ 7.2 (m, 4H, 2xAr-H), δ 2.0 (s, 6H, 2x-CH_3).

3.2. Synthesis of zinc complexes of 2-p-chloro-1H-imidazo[4,5-f][1,10]phenanthroline (4b)

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, 2xNH), δ 9.3 (dd, 4H, 2xH-phen), δ 8.9 (dd, 4H, 2xH-phen), δ 8.2 (m, 4H, 2xH-Ar), δ 7.9 (m, 4H, 2xH-Ar), δ 7.6 (dd, 4H, 2xH-phen).

3.3. Synthesis of zinc complexes of 2-(1H-indol-3-yl)-1H-imidazo [4, 5-f] [1, 10] phenanthroline (4c)

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; ^{1}H NMR (300 MHz, DMSO-d^6, δ ppm): δ 12.8 (s, 2H, 2xNH imidazole), δ 9.8 (s, 2H, 2xNH indole), δ 8.8 (dd, 4H,2xH-phen), δ 8.0 (dd, 4H, 2xH-phen), δ 7.5 (m,2H, 2xH-Ar), δ 7.2 (m, 2H, 2xH-Ar), δ 7.1 (m, 2H, 2xH-Pyrrole), δ 7.1 (dd, 4H, 2xH-phen), δ 6.8 (m, 2H, 2xH-Ar), δ 6.6 (m, 2H, 2xH-Ar).

3.4. Synthesis of zinc complexes of 2-(1H-7-Azaindol-3-yl)-1H-imidazo [4, 5-f] [1, 10] phenanthroline (4d)

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; ^{1}H NMR (300 MHz, DMSO-d^6, δ ppm): δ 13.4 (s, 2H, 2xNH-imidazole), δ 10.1 (s, 2H, 2xNH-indole), δ 8.9 (dd, 4H,2xH-phen), δ 8.5 (m, 2H, H-pyridyl), δ 8.0 (dd, 4H,2xH-phen), δ 7.6 (m, 2H, 2xH-pyridyl), δ 7.4 (m, 2H,2xH-pyridyl), δ 7.1 (dd, 4H, 2xH-phen), δ 6.5 (s, 2H, 2xH-Pyrrole).

Biological Activities

In *vitro* antimicrobial bioassay: The antimicrobial activities of the newly synthesized imidazo [4, 5-f] [1, 10] phenanthroline ligand and its complexes were screened in vitro for their antibacterial and antifungal activity against five bacterial and three fungal strains viz *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, *Trichoderma viridae* and *Aspergillus flavus* in DMSO solvent by agar diffusion method.[20, 21]

All the synthesized compounds were dissolved to prepare a stock solution of 1 mg/mL using 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 hrs at 37 ± 0.5 °C for antibacterial activity and 48 hrs 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

The analytical data and physical properties showed that, the imidazo [4, 5-f] [1, 10] phenanthroline derivatives and its complexes were light in color, stable and non hygroscopic at room temperature (table 1). The complexes were sparingly soluble in common organic solvents, but are readily soluble in strong coordinating solvents like DMF and DMSO. Decomposition points of the complexes are relatively high as these are stable up to temperature >300°C suggesting good thermal stability.[15] 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 newly synthesized compounds are summarized in the table 1.

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 1. From elemental analysis, the complex is of ML_2 type that is one central metal formed complex with two ligands.

Table 1. Elemental analysis (%C, %H, %N) and physical parameter data of the ligand and its complexes.

Compound	Mol. formula	Mol. Wt. g/mol	Color	Melting Point °C	% Yield	% Elemental Analysis					
						% C		% H		% N	
						Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
1	$C_{12}H_6N_2O_2$	210.19	Yellow	260	85	68.57	68.65	2.86	2.9	13.33	13.24
3a	$C_{20}H_{14}N_4$	310.35	Yellow	>300	75	77.42	77.21	4.52	4.51	18.06	18.14
3b	$C_{19}H_{11}ClN_4$	330.5	Bright Yellow	>300	55	68.99	68.94	3.33	3.41	16.94	17.14
3c	$C_{21}H_{13}N_5$	335	Bright Yellow	>300	62	75.22	75.75	3.88	4.01	20.9	19.95
3d	$C_{20}H_{12}N_6$	336	Bright Yellow	> 300	60	71.43	71.68	3.57	3.47	25	24.87
4a	$C_{40}H_{28}N_8Zn$	686	Yellow	>300	35	70.03	69.87	4.09	3.81	16.34	16.21
4b	$C_{38}H_{22}Cl_2N_8Zn$	726	Yellow	>300	47	62.78	62	3.03	2.89	15.42	15.55
4c	$C_{42}H_{26}N_{10}Zn$	735	Pale Yellow	> 300	42	68.54	69.07	3.54	3.61	19.04	19.21
4d	$C_{40}H_{24}N_{12}Zn$	737	Pale Yellow	>300	25	65.09	64.94	3.25	3.11	22.78	23.01

^{1}H-NMR spectra

The ^{1}H-NMR spectrum of the compound **1**, the signals at δ 8.97 ppm is corresponding to protons of 2 and 9 positions of quinoline ring. Also the signals at δ 7.65 ppm are due to the protons of 3 and 8 positions, while the peaks at δ 8.67 ppm are due to the protons of 4 and 7 positions.

In compounds 3a-3d the NMR signal at around δ 13.1 to 13.97 ppm is due to the -NH protons of imidazole ring, while the signals at δ 8.9-9.1 ppm, δ 8.0-8.96 ppm and δ 7.1-7.45 ppm are due to the protons of phenanthroline rings.

Also the signal at δ 2.32 ppm and the peaks at δ 8.16 ppm and at δ 7.95 ppm in compound 3a are due to the $–CH_3$ and aromatic protons respectively. In compound 3b, the NMR signals at δ 7.95 ppm and δ 8.16 ppm are due to aromatic protons.

In compounds 3c and 3d the signals at δ 9.91 ppm and δ 10.1ppm are due to the characteristic -NH of pyrrole nuclei of indole and azaindole respectively. Signals of aromatic protons are observed at aromatic region.

In compounds 4a-4d the NMR signals are similar like their ligands 3a-3d but slightly shifted to downfield or up field indicates coordinated ligand is in different environment due to rearrangement of protons as compared to free ligand molecule.[32] All compounds chemical shift value of PMR was summarized in table 2. Hence in 1H-imdazo [4, 5 –f] phenanthroline complexes new bonds may have formed by the involvement of lone pair of nitrogens.

^{13}C-NMR spectra

The ^{13}C-NMR spectrum of the compound 1, total six signals appeared as there is presence of six set of carbons. Since all carbons are of heteroaromatic 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 neighbouring electron donating nitrogen atom. Remaining signals appearing at aromatic region.

In ^{13}C-NMR spectrum of compounds 3a-3d there is absence of 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 3a the signal at δ 26.3 ppm is due to methyl carbon while remaining signals are much more similar to compound 1

and imidazole ring. Chemical shift values of CMR spectra of newly synthesized compounds were summarized in table 3.

Table 2. ^{1}H NMR signal in δ ppm

Compound	methyl protons	phenanthroline ring protons	Imidazole (-NH) protons	phenyl ring protons	Indole/Azaindole ring protons
1	-	7.65, 8.67, 8.97	-	-	-
3a	2.32	9.1, 8.96, 7.23	13.57,	8.16, 7.95	-
3b	-	9.10, 8.95, 7.45	13.97	8.05, 7.95	-
3c	-	9.1, 8.5, 7.3	13.1	-	9.91, 7.8, 7.7, 7.5, 7.2, 7.1.
3d	-	8.9, 8.0, 7.1	13.3	-	10.1, 8.5, 7.8, 7.4, 6.5
4a	2.00	9.3, 8.9, 7.5	13.1	8.0, 7.2	-
4b	-	9.3, 8.9, 7.6	12.9	8.2, 7.9	-
4c	-	8.8, 8.0, 7.1	12.8	-	9.8, 7.5, 7.2, 7.1, 6.8, 6.6
4d	-	8.9, 8.0, 7.1	12.9	-	10.1, 8.5, 7.6, 7.4, 6.5

Table 3. ^{13}C NMR signal in δ ppm

Compound	methyl carbons	phenanthroline ring carbons	imidazole ring carbon between 2 nitrogen atoms	phenyl ring carbons	Indole/Azaindole ring carbons
1	-	125.69, 129.57, 136.13, 152.76, 154.80, 178.22	-	-	-
3a	26.32	121.82, 122.42, 125.11, 129.25, 132.23, 143.96, 144.61, 154.77, 159.69	157.21	132.01,136.45, 140.19, 148.89	-
3b	-	122.7, 124.6, 126.8, 127.6, 129.0, 139.7, 142.6, 151.9, 162.7	161.9	129.8, 131.4, 131.9, 139.1	-
3c	-	121.8, 127.2, 129.0, 130.1, 131.7, 138.4, 142.3, 157.3, 159.9	158.1	-	111.8, 115.1, 119.6, 120.3, 125.9, 133.2, 137.9, 139.5
3d	-	121.8, 123.8, 126.0, 129.0, 130.1, 133.2, 137.4, 151.7, 158.1	157.3	-	101.8, 115.1, 120.3, 127.2, 131.7, 142.3, 146.8,

Scanning Electron Microscopy

The surface morphology of the complexes was studied by using SEM. The scanning electron micrographs were taken at 18 kV accelerating voltage and magnification was fixed according to need from 500x to 10000x. The uniform matrix was observed in the zinc metal complexes

4a-4d, shown in Fig. 2. From the SEM images, the complexes 4a and 4b shows the granular needle shaped morphology. However, SEM images of complexes 4c and 4d showing uniform granular rod shaped morphology. Heterocyclic ring in the imidazo- phenanthroline ligand may influence on the morphology.

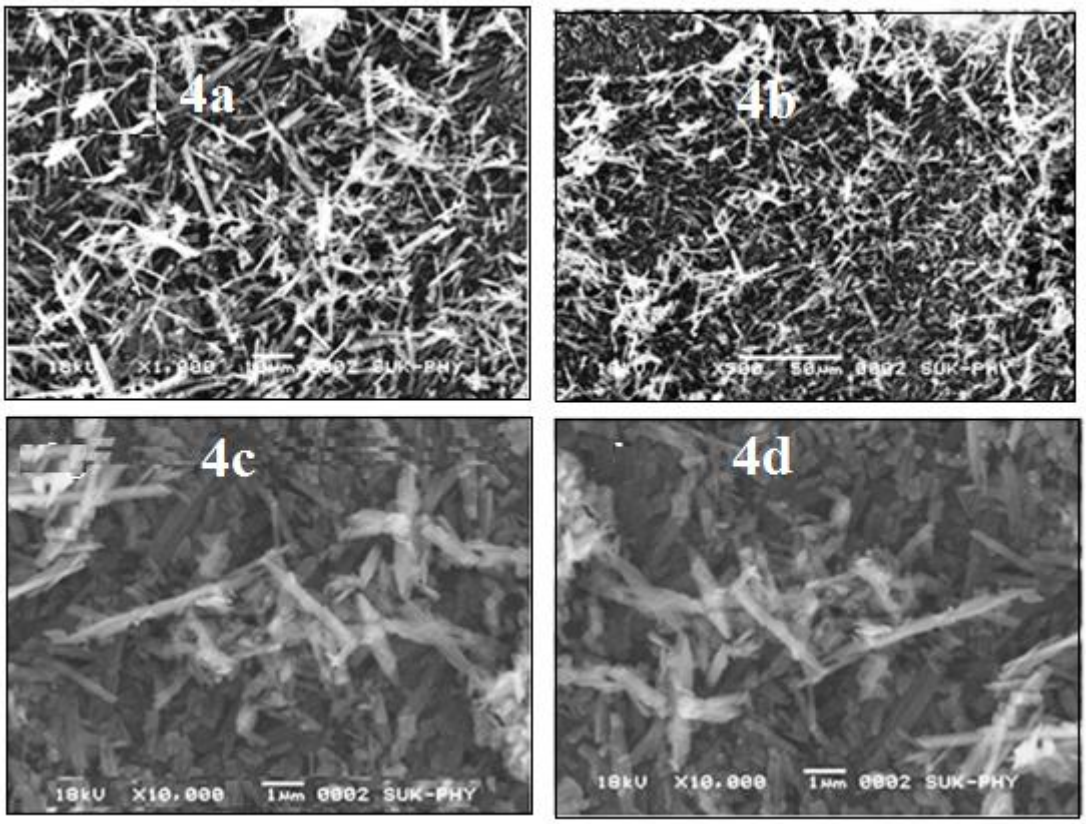


Figure 2: SEM images of zinc complexes 4a, 4b, 4c and 4d.

Biological Screening

Antimicrobial activity (agar diffusion method)

In this study, newly synthesized phenanthroline derivatives were subjected to antimicrobial study by *in vitro* disk diffusion method against bacterial and fungal strains. 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. The results are reported as a mean of triplicate measurements.

Antibacterial and antifungal activities of synthesized compounds are shown in table 4. Results shows 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 3c, 3d, 4a-4d are active against both gram positive and gram negative strains, whereas as some compounds viz.1, 3a and 3b are inactive against selected bacterial strains. Compounds 4a, 4b, 4c and 4d have shown excellent antibacterial activity as compared to that of standard at tested concentration. Interestingly, compounds 3a, 3b, 3c and 3d showing moderate antibacterial activity. The difference in activities is due to the presence of zinc metal in 4a-4d. 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.

Antifungal activities of newly synthesized compounds are also shown in table 4. Compounds 3b, 3c, 3d, 4a, 4b, 4c, and 4d 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 substituents.

Table 4: Antimicrobial Results

Antimicrobial activity of ligands and their Zn complexes (zone of inhibition in mm)

Compound	Bacteria (MIC at 50 µg/mL)					Fungi (MIC at 100µg/mL)		
	EC	KN	PV	SA	BS	AN	TV	AF
1	5	ND	ND	8	9	7	ND	11
3a	7	10	ND	6	10	ND	11	9
3b	8	ND	7	6	ND	11	4	8
3c	9	8	11	9	8	10	14	10
3d	8	11	10	12	9	8	11	10
4a	12	13	6	10	11	8	11	8
4b	12	13	6	10	11	9	15	10
4c	10	18	15	13	14	14	12	12
4d	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

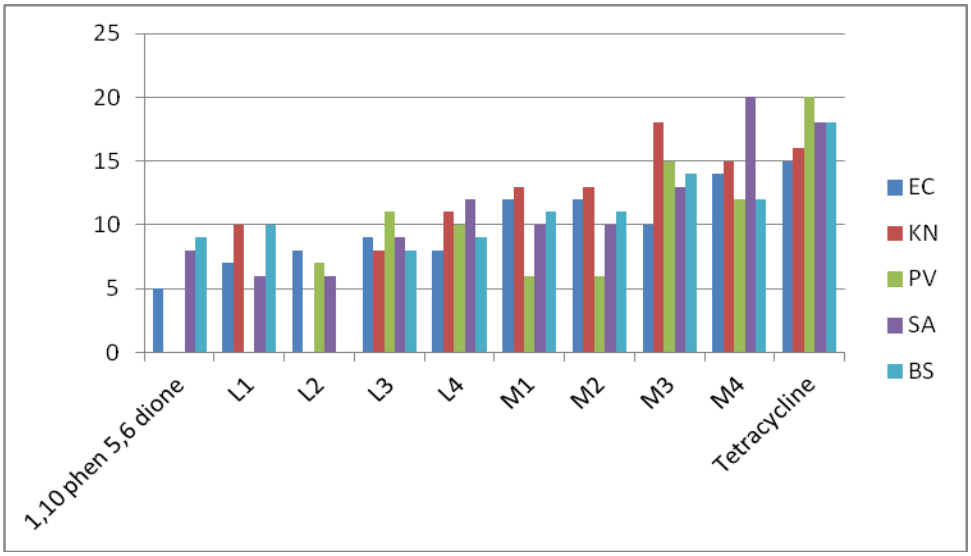


Figure 3: Comparative study of inhibition of bacterial growth.

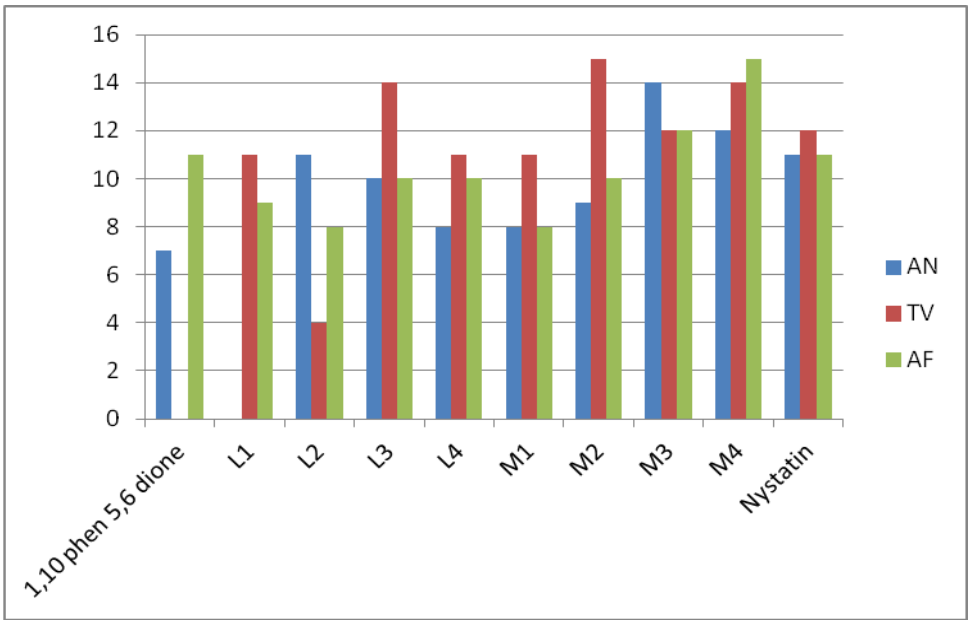


Figure 4: Comparative study of inhibition of fungal growth.

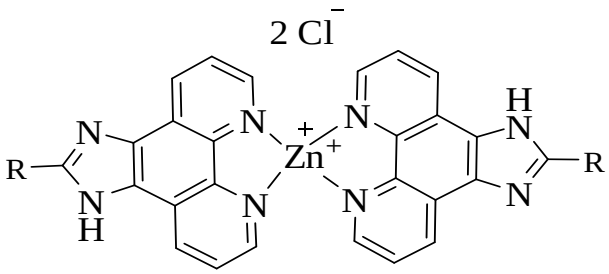


Figure 5: Proposed Structures of Zn (II) complexes, where R = aromatic or heterocyclic aldehyde.

CONCLUSION

In conclusion, we describe the synthesis of new bidentate imidazo phenanthroline ligands and their zinc complexes. The synthesized ligand and its metal complexes individually exhibited varying degrees of inhibitory effects on the growth of the tested bacterial and fungal species. The antimicrobial results evidently show that the activity of the imidazo phenanthroline compounds became more pronounced when coordinated to the zinc metal ion. Hence based upon above findings the proposed structure of the imidazo phenanthroline- zinc complexes is shown in figure 5.

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