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Synthesis of ternary metal complexes of bivalent metal ions with benzimidazole derivative and their antimicrobial studies

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A rapid, conventional reflux and efficient procedure is used to synthesize a series of ternary metal complexes derived from Schiff base of 2-aminobenzimidazole with 2-chloroacetophenone and secondary ligands (8-hydroxy quinoline or L-histidine). Our present work focuses on the potential of the synthesized Schiff base, its metal complexes and the study of their antimicrobial activity. All these compounds are characterized by elemental analysis, melting point, magnetic moment measurement, molar conductance measurement and various spectral techniques such as FTIR, ^{1}H NMR, and mass spectra. Among these synthesized complexes, Ni(II) complex having 8-hydroxyquinoline as a secondary ligand exhibits excellent antifungal activity against *Aspergillus niger* compared to standard Ketoconazole whereas other metal complexes show good to moderate activity against gram-positive bacteria *i.e. Staphylococcus aureus* and gram-negative bacteria *i.e. Escherichia coli* compared to standard Ciprofloxacin.

Keywords: Benzimidazole, Acetophenone, 8-Hydroxyquinoline, Histidine, Ternary metal complex, Antimicrobial activity

In the present era, many infectious and contagious diseases have spread due to the appearance of various multidrug-resistant bacterial and fungal strains around the world, which are resistant to antimicrobial agents having some mutation in their own structure, so there require some drugs and medicine to prevent them. Heterocyclic compounds containing N, O and S are effective against various microorganisms.

Benzimidazoles are essential heterocyclic compounds for developing various biological and pharmaceutical research molecules. These compound's structure is similar to purine, so these also have an antibacterial ability due to the inhibition of the synthesis of proteins and nucleic acid inside the bacterial body structure. Benzimidazole moiety is more attentive to researchers for drug designing because various drugs such as albendazole (anthelmintic), omeprazole (antiulcer) and telmisartan (antihypertensive) contain benzimidazole as a core template[1]. Benzimidazole derivatives exhibiting various biological activities like anticancer[2], antimicrobial[3], anti-inflammatory[4], antihistaminic[5], antioxidant[6], antiviral[7], antidiabetic[8], antifungal[9] and antihypertensive[10], *etc*. have made it an indispensable anchor for the development of new therapeutic agents. Previous literature suggested that substituted acetophenone was also a bioactive compound containing various activities such as antimicrobial[11], anti-inflammatory[12] and anticancer[13] activities, along with some other useful bioactive applications.

Sometimes two or more heterocyclic compounds when combined result in a new molecule which could attain a novel moiety with increased bioactivities. Metal complexes or ternary complexes are generally more potent than those corresponding to their free ligand[14,15].

Owing to such a broad range of biological potential, it is highly attractive for researchers to synthesize and explore the biological performance of new benzimidazole compounds.

Experimental Section

Materials and methods

All the solvents, reagents and starting materials (2-aminobenzimidazole, 2-chloroacetophenone, 8-hydroxyquinoline and L-histidine) were of the highest purity and were obtained by commercially suppliers of Sigma Aldrich and also were used without any purification. The progress of the reaction was checked by thin layer chromatography (TLC), which was obtained from Merck (pre-coated silica-gel, aluminium plates), using appropriate developing solvent and visualization in iodine chamber and UV chamber. Melting points were determined by open

capillary electrothermal apparatus. IR spectra were recorded on Perkin-Elmer spectrometer in 4000 cm^{-1} - 400 cm^{-1} range and ^1H NMR spectra were obtained in DMSO-d_6 solution with a Jeol Resonance ECS-400 Spectrometer at 400 MHz. Mass spectra were taken in TOF MS ES^+ mass spectrometer operating at an ionization potential of 70 eV.

Synthesis of Schiff base ligand (L)-[N-(1*H*-benzo[d]imidazol-2-yl)-1-(2-chlorophenyl)ethan-1-imine]

Our previously reported method[3] was used for the synthesis of this ligand, an ethanolic solution of 2-aminobenzimidazole (1.33 g, 10 mmol) added with ethanolic solution of 2-chloroacetophenone (1.54 g, 10 mmol) into 250 mL round bottom flask and allowed to reflux on heating mantle. A few drops of glacial acetic acid and a pinch of sodium acetate were added to the reaction mixture. Light brown colour of reaction mixture was obtained after ~6 hours of reflux. Completion of the reaction was monitored by TLC using a suitable solvent. Then the mixture was allowed to cool at RT and kept overnight. After that light brown solid product was obtained. Further, washed with ethanol, recrystallized with ethanol, and dried in desiccator over silica (Scheme 1).

General procedure of compounds (A-C)

An aqueous solution of metal acetate salt [Ni(II), Fe(II)or Zn(II)] was added to a hot ethanolic solution of synthesized ligand L (2.69 g, 10 mmol) in 250 mL round bottom flask, allowed to reflux. After one hour, 8-hydroxyquinoline as a secondary ligand L_1 (1.45 g, 10 mmol) was added and reflux continued for ~5-hours. TLC indicated the completion of the reaction and purity of the compounds. After then, the reaction mixture is concentrated *via* slow evaporation of solvent at RT. The obtained solid product was washed, recrystallized with ethanol, and dried in a desiccator over silica (Scheme 2).

General synthetic procedure of compounds (D-F)

The same procedure was employed for synthesizing the complexes as mentioned above, where L-histidine (L_2) was used to replace 8-hydroxyquinoline (Scheme 3).

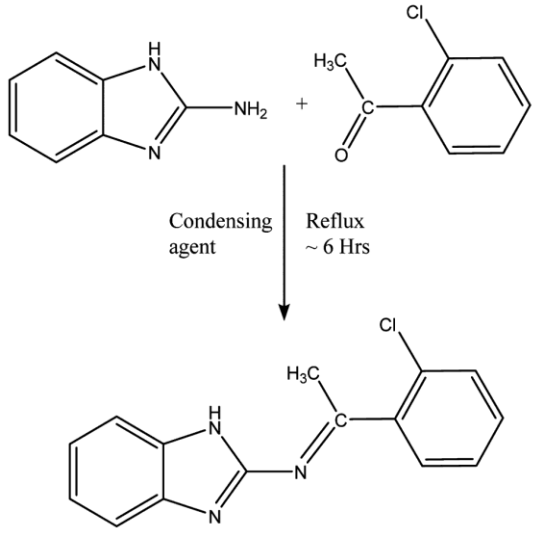
Physicochemical and spectral characterization

Ligand [N-(1*H*-benzo[d]imidazol-2-yl)-1-(2-chlorophenyl)ethan-1-imine]

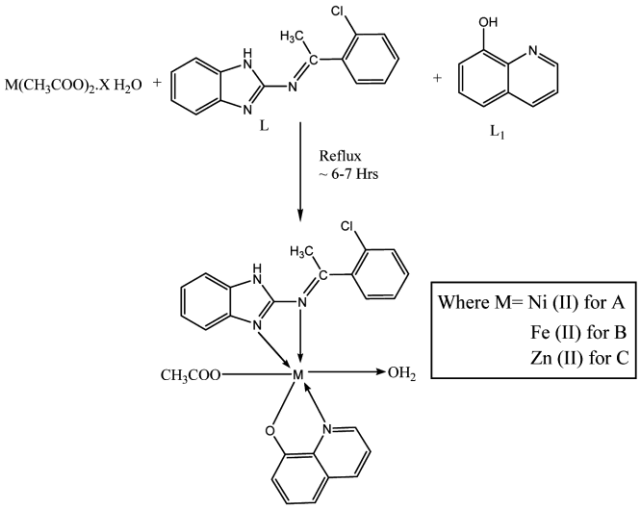
Yield 78.54%. Brown colour. m.p. ~194.2°C. Elemental analysis [$C_{15}H_{12}N_3Cl$] Calcd: C, 66.76; H, 4.49; N, 15.58; Cl, 13.17. Found: C, 66.21; H, 4.14; N, 15.71; Cl, 13.85%. IR: 1425 (C-C_{str}), 780 (C-C_{bend}), 1561 (C=N), 2970 cm^{-1} (C-H_{str}); ^1H NMR (400 MHz, DMSO-d_6): δ =8.3 (N-H, Benzimidazole), 1.8 (CH_3, azomethine), 7.1 -7.8 (Ar-H); MS (70 eV): Calcd for [$C_{15}H_{12}N_3Cl$]: *m/z* 234.22[M-Cl]$^+$.

Compound A, [Ni(L)(8-HQ)(OAc)(H_2O)]

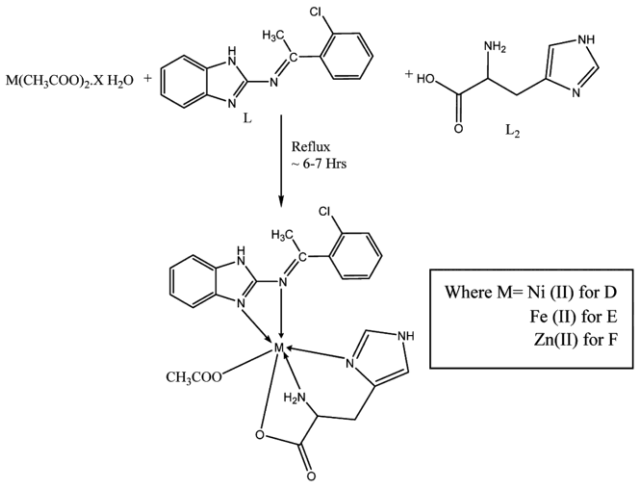
Yield 57.18%. Light green colour. m.p. ~211.4°C. Elemental analysis [$C_{26}H_{23}N_4O_4ClNi$] Calcd: C, 56.79; H, 4.22; N, 10.19; Cl, 6.46; Ni, 10.68. Found: C, 56.24; H, 4.86; N, 10.74; Cl, 6.13; Ni,10.23%. IR: 1405 (C-C_{str}), 739 (C-C_{bend}), 1536 (C=N), 3078 (C-H_{str}), 1608 (C=O), 604 (M-O), 461 cm^{-1} (M-N);



Scheme 1 — Plausible synthetic route of synthesized Schiff base ligand (L)



Scheme 2 — Proposed structure of mixed ligand complexes (A-C) with 8-hydroxyquinoline



Scheme 3 — Proposed structure of mixed ligand complexes (D-F) with L-Histidine

^{1}H NMR (400 MHz, DMSO-d_6): δ =8.0 (N-H, Benzimidazole), 2.3 (CH_3, acetic acid), 1.77 (CH_3, azomethine), 7.1 -7.8 (Ar-H), 3.2 (H_2O); MS (70 eV): Calcd for $[C_{26}H_{23}N_4O_4ClNi]$: *m/z* 512.62$[M-Cl]^+$.

Compound B, [Fe(L)(8-HQ)(OAc)(H_2O)]

Yield 71.85%. Black colour. m.p. ~202.8°C. Elemental analysis $[C_{26}H_{23}N_4O_4ClFe]$ Calcd: C, 57.09; H, 4.24; N, 10.25; Cl, 6.50; Fe, 10.22. Found: C, 57.18; H, 4.63; N, 10.67; Cl, 6.21; Fe, 10.43%. IR: 1411 (C-C_{str}), 751 (C-C_{bend}), 1552 (C=N), 3024 (C-H_{str}), 1631 (C=O), 616 (M-O), 447 cm^{-1} (M-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.56 (N-H, Benzimidazole), 2.3 (CH_3, acetic acid), 1.8 (CH_3, azomethine), 6.8-8.2 (Ar-H), H_2O= 3.0; MS (70 eV): Calcd for $[C_{26}H_{23}N_4O_4ClFe]$: *m/z* 513.47$[M-Cl-2]^+$.

Compound C, [Zn(L)(8-HQ)(OAc)(H_2O)]

Yield 52.43%. Light greenish-yellow colour. m.p. ~218.5°C. Elemental analysis $[C_{26}H_{23}N_4O_4ClZn]$ Calcd: C, 56.11; H, 4.17; N, 10.07; Cl, 6.38; Zn, 11.76. Found: C, 56.28; H,4.13; N, 10.62; Cl, 6.11; Zn, 11.43%. IR: 1418 (C-C_{str}), 746 (C-C_{bend}), 1544 (C=N), 3074 (C-H_{str}), 1621 (C=O), 601 (M-O), 433 cm^{-1} (M-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ =8.8 (N-H, Benzimidazole), 2.4 (CH_3, acetic acid), 1.5 (CH_3, azomethine), 6.2- 7.2 (Ar-H), H_2O= 3.2; MS (70 eV): Calcd for $[C_{26}H_{23}N_4O_4ClZn]$: *m/z* 518.78[M-Cl-1]$^+$.

Compound D, [Ni(L)(His)(OAc)]

Yield 58.39%. Light green colour. m.p. ~231.8°C. Elemental analysis $[C_{23}H_{23}N_6O_4ClNi]$ Calcd: C, 50.98; H, 4.28; N, 15.52; C, 6.56; Ni, 10.84. Found: C, 50.28; H, 4.13; N, 15.62; Cl, 6.13; Ni, 10.21%. IR: 1435 (C-C_{str}), 781 (C-C_{bend}), 1554 (C=N), 2991 (C-H_{str}), 1618 (C=O), 623 (M-O), 451 cm^{-1} (M-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ =7.9 (N-H, Benzimidazole), 2.8 (CH_3, acetic acid), 1.9 (CH_3, azomethine), 5.8 (NH_2 Histidine), 6.6-7.1 (Ar-H), 2.1 (CH_2, Histidine side chain); MS (70 eV): Calcd for $[C_{23}H_{23}N_6O_4ClNi]$: *m/z* 504.78$[M-Cl]^+$.

Compound E, [Fe(L)(His)(OAc)]

Yield 68.62%. Dark-brown colour. m.p. ~226.3°C. Elemental analysis $[C_{23}H_{23}N_6O_4ClFe]$ Calcd: C, 51.25; H, 4.31; N, 15.60; Cl, 6.59; Fe, 10.37. Found: C, 51.38; H, 4.19; N, 15.22; Cl, 6.17; Fe, 10.28%. IR: 1420 (C-C_{str}), 748 (C-C_{bend}), 1541 (C=N), 3012 (C-H_{str}), 1631 (C=O), 595 (M-O), 452 cm^{-1} (M-N); NMR (400 MHz, DMSO-d_6): δ =8.0 (N-H, Benzimidazole), 3.4 (CH_3, acetic acid), 1.7 (CH_3, azomethine),6.0 (NH_2, Histidine), 6.6-7.2 (Ar-H), 1.1 (CH_2, Histidine side chain); MS (70 eV) Calcd for $[C_{23}H_{23}N_6O_4ClFe]$: *m/z* 502.78$[M-Cl]^+$.

Compound F, [Zn(L)(His)(OAc)]

Yield 65.87%. Light brown colour. m.p. ~238.9°C. Elemental analysis $[C_{23}H_{23}N_6O_4ClZn]$ Calcd: C, 50.36; H, 4.23; N, 15.33; Cl, 6.48; Zn, 11.92. Found: C, 50.71; H, 4.16; N, 15.28; Cl, 6.12; Zn, 11.20%. IR: 1395 (C-C_{str}), 782 (C-C_{bend}), 1542 (C=N), 2993 (C-H_{str}), 1622 (C=O), 592 (M-O), 439 cm^{-1} (M-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ =7.7 (N-H, Benzimidazole), 2.4 (CH_3, acetic acid), 1.8 (CH_3, azomethine), 5.3 (NH_2, Histidine), 6.8-7.0 (Ar-H), 2.9 (CH_2, Histidine side chain); MS (70eV): Calcd for $[C_{23}H_{23}N_6O_4ClZn]$: *m/z* 513.21$[M-Cl]^+$.

Antimicrobial study

In vitro antibacterial activity of the newly synthesized compounds against two bacterial strains, *Staphylococcus aureus* and *Escherichia coli*, were investigated by the agar well diffusion method using Mueller Hinton agar (Hi Media, India) as a bacteriological medium at four different concentrations 20, 40, 60 and 80 µg/mLin comparison with that of commercially available control drug Ciprofloxacin. These compounds were diluted in DMSO (10mg/mL) after solidifying agar medium (Mueller Hinton) in Petri-dishes. The antimicrobial strains to be tested were injected on the agar medium surface. The plates were placed well and incubated at 37°C for 24 hours; the inhibition zone formed was calculated in millimetres.

The antifungal analysis was evaluated against the fungal strain *Aspergillus niger* using agar well

Table 1 — Results of antimicrobial activity (MIC values) of compounds

Compd	Bacterial strain		Fungal strain
	Gram +ve	Gram –ve	
	S. aureus	*E. coli*	*A. niger*
	20 40 60 80 (μg/mL)	20 40 60 80 (μg/mL)	20 40 60 80 (μg/mL)
(L)[$C_{15}H_{12}N_3Cl$]	N N N 9	8 10 12 14	N 8 12 13
A[Ni(L)(8-HQ)(OAc)(H_2O)]	14 16 18 19	13 15 17 19	18 20 21 24
B[Fe(L)(8-HQ)(OAc)(H_2O)]	14 15 16 18	12 13 17 19	N 12 14 17
C[Zn(L)(8-HQ)(OAc)(H_2O)]	11 13 15 16	9 11 13 14	N N 10 12
D[Ni(L)(His)(OAc)]	N N 10 12	N N N 10	N N N N
E[Fe(L)(His)(OAc)]	N N 8 11	N N 8 11	N N N 8
F[Zn(L)(His)(OAc)]	11 12 14 15	9 10 12 14	N N 8 11
Standard (40 μg/mL) Ciprofloxacine	30	30	25
Ketoconazole			

diffusion method and using Sabouraud's dextrose agar (Merck, Germany) as a cultural media and compared with standard Ketokenazole control drug.

The outcome of the antimicrobial bioactivity of the compounds, as mentioned earlier and their MIC values are shown in Table 1.

Results and Discussion

Physicochemical analysis of synthesized ligand and its metal complexes revealed that these are coloured and soluble in methanol, ethanol, DMF and DMSO. Molar conductance of complexes calculated in DMSO were found ~10- 13 $\Omega^{-1}cm^2mol^{-1,}$ which show the non-electrolytic behaviour of the complexes. Magnetic moment of the complexes obtained 3.24B.M., 4.98B.M. and negligible for Ni(II), Fe(II) and Zn(II) complexes, respectively, which indicates the paramagnetic nature of Ni and Fe metal ions and diamagnetic nature of Zn metal ion due to having unpaired electrons 2, 4 and 0 respectively.

UV-Vis spectra of the synthesized compounds were recorded in ethanol in the range of 200-800 nm. Ligand shows two absorption maxima at 285nm for n-π* and 298 nm for π-π*, which are shifted towards high wavelength in the metal complexes that indicate the formation of metal-ligand bonds in metal complexes.

FTIR spectra of ligand and metal complexes show that the absorption band of azomethine bond (C=N) of ligand (υ = 1561cm^{-1}) shifted to lower frequencies in metal complexes, confirming the coordinate bond between metal ions and azomethine. Some new absorption bands shown in the range 425cm^{-1} to 620cm^{-1} correspond to M-N and M-O coordinate bonds, respectively.

^{1}H NMR spectra of synthesized metal complexes were recorded in DMSO-d_6 solvent. Herein, we observed NMR signals for various types of H-atoms present in the complexes that define the metal complexes fine structure[16] (Fig. S1-S6, Supplementary Information).

The mass spectra presented the molecular weight of the synthesized ligand and its metal complexes; hence it is the evidence of the synthesis of new compounds. The MS ESI +ve of the synthesized ligand (L) shows the parent ion peak *m/z* at 234.22 for $[M\text{-}Cl]^+$, which is very close to the molecular weight (234.09) of the synthesized ligand $C_{15}H_{12}N_3Cl$. In addition, some other prominent peaks were observed at $[M+1]^+$ = 134.14, 146.14 for fragments of the ligand, which is agreed with the plausible structure of the Schiff base ligand. Thus, metal complexes also have shown the parent ion peak *m/z* at 512.62, 513.47, 518.78 & 513.21 for $[M\text{-}Cl]^+$ of [Ni(L)(8-HQ)(OAc)(H_2O)], [Fe(L)(8-HQ)(OAc)(H_2O)], [Zn(L)(8-HQ)(OAc)] and [Zn(L)(His)(OAc)] metal complexes, respectively and some other prominent peaks also seen. Mass spectra of selected compounds are shown in Fig.S7-S11 (Supplementary Information).

The results suggested that Schiff base has low efficacy against bacterial and fungal strains, but their metal complexes exhibit moderated to excellent efficacy against bacterial and fungal strains. The increase in the efficacy of the metal compounds against microorganisms is clarified by overtone's concept of cell permeability and chelation theory[17]. According to the above theory, in the complexes, polarity of the metal ions is reduced by coordination of ligands due to sharing the positive charge of metal ions with donor atom of ligands enclosed with the

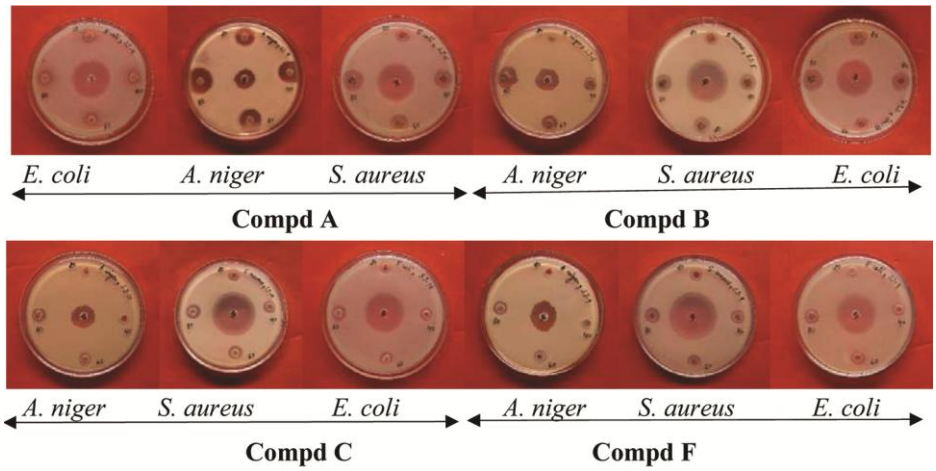


Fig. 1 — Antimicrobial studies of selected synthesized compounds

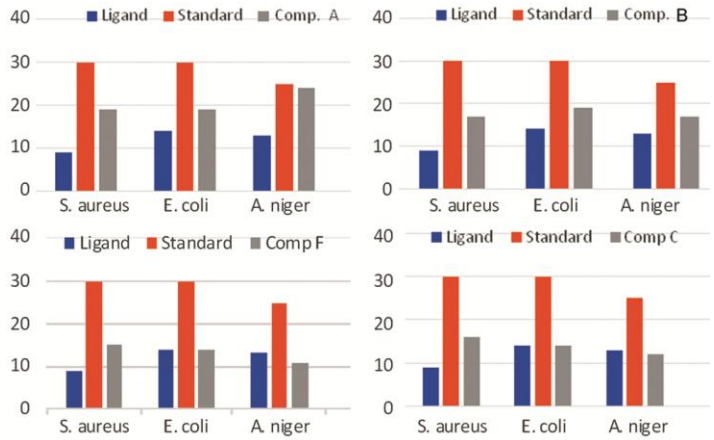


Fig. 2 — Bar diagram of the maximum inhibitory value of the antimicrobial activity of ligand (L) and selected metal complexes

chelating system. Thus, the increased lipophilic nature of metal chelates supported its permeation to lipid membrane of microorganisms more effectively and destroyed them more rapidly[18].

The biological activity is not only affected by chelating but also affected by various factors such as solubility, the redox potential of metal ions, dipole moment, size, geometry of the complexes, coordination sites and bond length between metal and donor atom of ligand[19].

Most of the compounds show good antimicrobial activity except compound D [Ni(II)] and compound E [Fe (II)], both having Histidine as a secondary ligand. Compound A, which has Ni(II) metal as a central atom, is most effective against all three selective pathogens and Compound B [Fe(II)] has also shown promising activity. Compound C and Compound F both have Zn(II) metal as a central atom, show moderate activity against both bacterial strains but are less effective for *A. niger*. Compound A has better potency as compared to standard control drug against fungal strain *A. niger* (Fig. 1, Fig. 2).

Conclusion

Newly synthesized Schiff base ligand using 2-aminobenzimidazole with 2-chloroacetophenone, further allowed to complexation with Fe(II), Ni(II), and Zn(II) metal and 8-hydroxyquinoline (bidentate) or L-histidine (tridentate) as a secondary ligand. These Ni(II), Fe(II) and Zn(II) complexes have been proposed to exhibit octahedral geometry which is determined with various spectral techniques. The ligand, L as bidentate, is coordinated to three different metals in different complexes *via* azomethine nitrogen atom and nitrogen of imidazole ring. These complexes exhibited good to excellent biological significance against bacterial and fungal strain. Ni (II) and Fe(II) complexes of 8-hydroxyquinoline are most

effective against both bacterial strain *S. aureus* and *E. coli* as well as fungal strain *A. niger*. Secondary ligand histidine-containing complexes are generally less effective compared to 8-hydroxyquinoline ligand. Among all the metal complexes, most of the complexes exhibit a high capacity to inhibit growth of microbes compared to free ligand. As suggested, this synthesized Schiff base and its complexes can be used as a good controller against microbes and their substituent also influence their bioactivity, so by changing many substituents on benzimidazole and acetophenone; those can be used as antimicrobial agents with good effectiveness compared to standard drugs.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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Conflict of interest

The authors declare no conflict of interest.

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