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Synthesis, Characterization and Antimicrobial Properties of Mannich Base Cyclization Derivatives of Benzimidazole and Their Metal Complexes

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Abstract Novel Mannich base cyclization derivatives of Benzimidazole were prepared through three-component condensation reaction of 2-aminobenzimidazole with formaldehyde and primary amines. All the compounds were characterized through spectral and analytical data. The transition metal complexes of resultant Mannich bases have been synthesized and well characterized by elemental analyses, spectral studies, magnetic moment determination, molar conductivity measurement and Thermogravimetric analysis. Experimental results showed that metal complexes act as bi-dentate ligands. The in-vitro antibacterial and antifungal activity of Mannich bases and their metal (II) complexes was assayed against different pathogens using MIC method. All the compounds and their metal complexes showed good potency against various microorganisms. The synthesized compounds and their metal complexes were also screened for their cytotoxicity and results showed that only Ni (II) complexes exhibit cytotoxicity while all other compounds were almost inactive.

Keywords Mannich bases, Benzimidazole, Metal complexes, Anti-microbial agents

1. Introduction

The benzimidazoles contain a phenyl ring fused to an imidazole ring. Benzimidazole and their derivatives have diverse applications in coordination chemistry, photophysics, photochemistry and bioinorganic chemistry. [1-4] Three component condensation reaction of Benzimidazole is very important for the synthesis of various useful compounds.[5] Over the past few decades, Mannich base reactions of benzimidazole have been the guiding tent for the synthetic chemists because of their widespread pharmaceutical importance i.e. antibacterial[6],anthelmintic [7], antifungal[8], anti-inflamatory[9], antiviral [10] and analgesic[11] properties. In addition to their biological importance, benzimidazoles form stable complexes with various transition metals.[12] Transition metal complexes of 2-substituted benzimidazole and benzimidazole-based mixed ligands have been reported with mono-, bi- and tri-dentate coordination behavior.[13-17] Continuous increase in bacterial resistance to existing drugs has been resulted due to wide spread use of antibacterial agents leading to research on new substances possessing antimicrobial activity.[18,19] Several benzimidazoles are commercially available as pharmaceuticals, veterinary products and fungicides.

The worthwhile biological activities of Mannich bases have been guiding for the synthesis of novel Mannich bases. The main objective of present communication is to provide a comprehensive account of N-Mannich type bases of benzimidazole, their chelating behavior and to highlight their potential in evolving better antimicrobial drugs. A total of 3 Mannich bases and 12 metal(II) complexes have been prepared in this study and well characterized by their physical, spectral and analytical data. The synthesized compounds were further evaluated for their antimicrobial properties against various pathogens using MIC method.

2. Experimental Work

2.1. General Manipulations

All the reagents and solvents were purchased from Sigma-Aldrich and they were used as received. Reactions were monitored by thin layer chromatography (plates coated with 0.2 mm Merck 60 F254 silica gel) and were visualized by UV irradiation (254 nm). Elemental analyses were carried out with a LECO-CHNS-9320 model. ^{1}H-NMR spectra of

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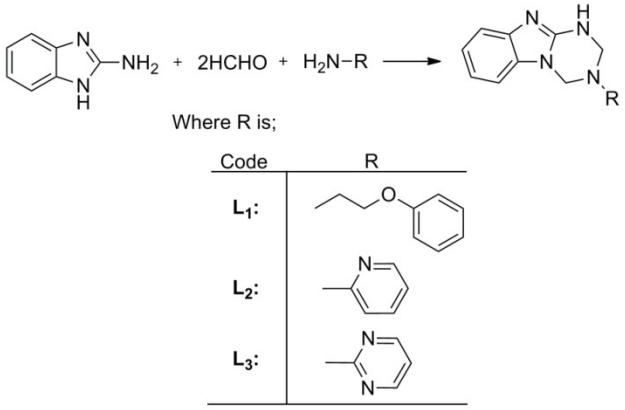
compounds were recorded with a Bruker Spectrospin Avance DPX-300 using TMS as internal standard and d_6 DMSO as solvent. Infrared spectra of compounds were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer. The melting points of compounds were determined with a Gallenkamp melting point apparatus. UV/visible absorption spectra were recorded using a Shimadzu UV-1700 spectrophotometer at room temperature. Conductance was recorded by pre-calibrated cyber scan 500 conductivity meter. Electron impact mass spectra (EIMS) were recorded on a JEOL MS Route instrument. Thermogravimetric analysis (TGA) was carried out under constant nitrogen flow at a heating rate of 15°C min^{-1}, using a Mettler Toledo TGA/SDTA 851 balance. The heating scans were performed on 3-5 mg of sample, in the temperature range 25-900°C. In vitro antibacterial, antifungal and cytotoxic properties were studied at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

2.2. Synthesis of Mannich Bases (Scheme 1)

To a solution of 2-aminobenzimidazole (0.05 mole) in 30 ml of 1-4dioxane, 0.1 mole formaldehyde and 0.05 mole ethanolic solution of respective primary amine were added. The mixture was stir for 2 h at 75°C. A clear solution was obtained. The completion of reaction was monitored by TLC. The obtained solution was filtered and reduced to half of its volume by evaporation of the solvent in vacuo. The concentrated solution was left overnight at room temperature, which led to the formation of a solid product. This solution was filtered, washed with dioxane then with ether and, dried.



Scheme 1. Synthesis of Mannich bases

2.3. Synthesis of Metal Complexes (Scheme 2)

To a hot magnetically stirred methanolic solution of Mannich bases (L_1-L_3) (0.1 mole), a methanolic solution of metal(II) salts (0.05 mole) was added. The mixture was then refluxed for 2 h. A clear solution was obtained. The completion of reaction was monitored by TLC. The solution obtained was cooled at room temperature, precipitates appeared were filtered and washed with acetone and dried.

Where;
M = Co, Ni, Cu, Zn
L = L_1, L_2, L_3

Scheme 2. Synthesis of metal complexes

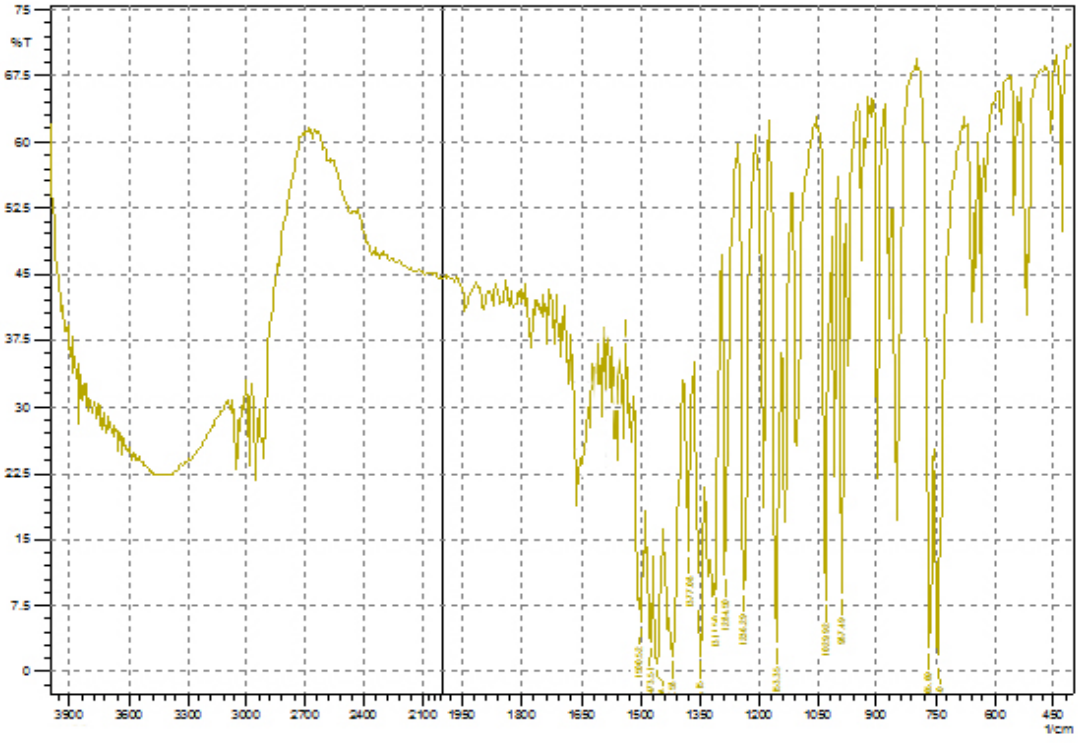


Figure 1. Representative IR spectra of L_1

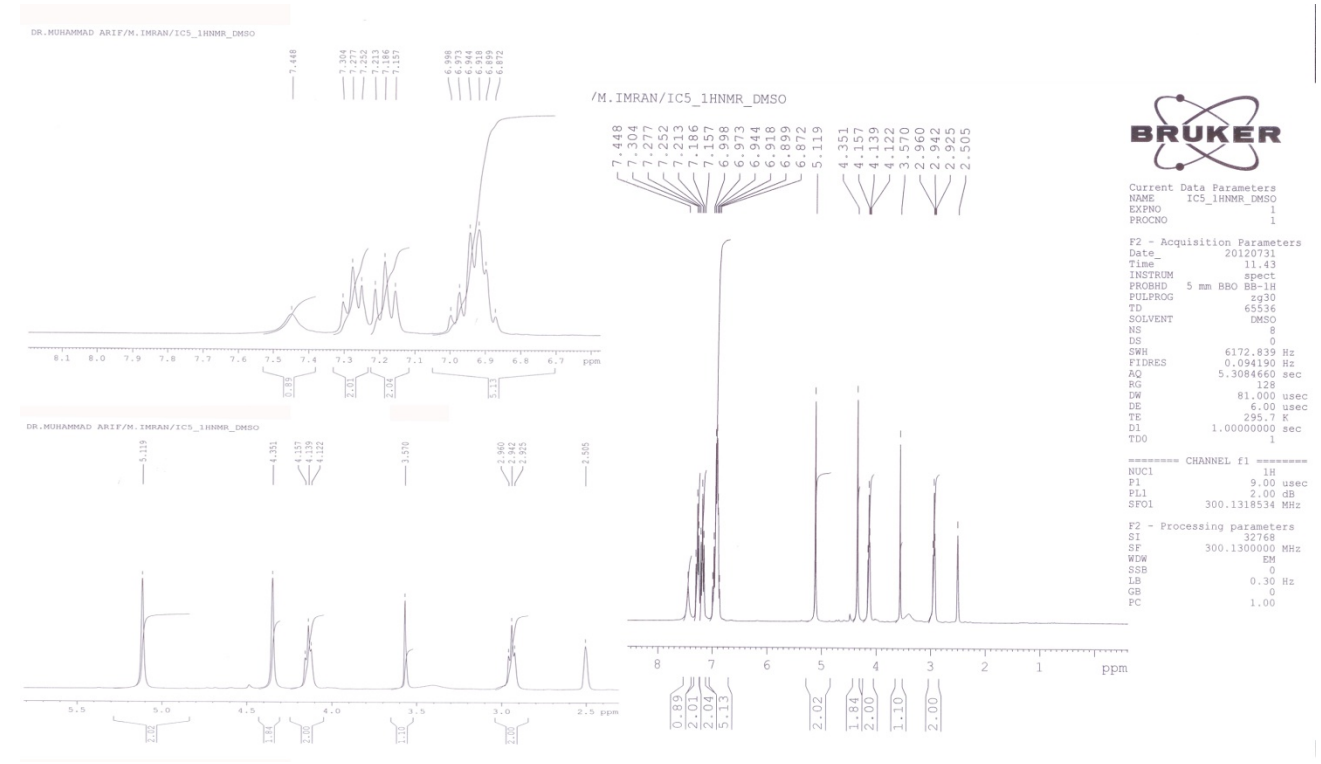


Figure 2. Representative ^{1}NMR Spectra of L_1

2.4. Compound 1 (L_1): 3-(2-phenoxyethyl)-1,2,3,4-tetrahydrobenzo[4,5] Imidazo[1,2-a][1,3,5] Triazine

White solid; yield 56%; m.p: 175°C; IR (KBr): 3382 (NH stretching), 3055 (Aromatic CH_2), 2847 (CH_2 Stretching), 1655 (C=N), 1355 (C-N, Amine), 1242 (C-O); ^{1}H-NMR (DMSO-d_6): δ 7.2-7.3 (t, 2H, benzimidazole ring), 7.1-7.2 (t, 2H, benzimidazole ring), 6.8-6.9 (m, 5H, phenyl ring), 5.1 (s, 2H, N-CH_2-N), 4.3 (s, 2H, N-CH_2-NH), 4.1 (t, 2H, -O-CH_2), 3.6 (s, 1H, NH), 2.9 (t, 2H, N-CH_2-C); Mass spectrum (ESI) [M]+ = 294; Anal. Calcd. for $C_{17}H_{18}N_4O$ (294.35) (%): C, 69.37; H, 6.16; N, 19.03. Found (%): C, 69.30; H, 6.12; N, 19.08. ^{1}H-NMR of Zn(II) complex (DMSO-d_6): δ 7.4-7.5 (t, 2H, benzimidazole ring), 7.3-7.4 (t, 2H, benzimidazole ring), 7.1-7.3 (m, 5H, phenyl ring), 5.3 (s, 2H, N-CH_2-N), 4.5 (s, 2H, N-CH_2-NH), 4.3 (t, 2H, -O-CH_2), 3.8 (s, 1H, NH), 3.1 (t, 2H, N-CH_2-C).

2.5. Compound 2 (L_2): 3-(pyridin-2-yl)-1,2,3,4-tetrahydrobenzo[4,5] Imidazo[1,2-a][1,3,5] Triazine

White solid; yield 42%; m.p: 193°C; IR (KBr): 3382 (NH stretching), 3055 (Aromatic CH_2), 2854 (CH_2 Stretching), 1660 (C=N), 1574 (NH bending), 1348 (C-N, aromatic amine); ^{1}H-NMR (DMSO-d_6): δ 8.0 (d, 1H, pyridine ring), 7.3-7.4 (m, 2H, benzimidazole ring), 7.1 (d, 1H, pyridine ring), 6.9 (m, 2H, benzimidazole ring), 6.8 (s, 2H, N-CH_2-N), 6.5-6.6 (m, 2H, pyridine ring), 5.4 (d, 2H, N-CH_2-NH), 3.6 (s, 1H, NH); Mass spectrum (ESI) [M]+ = 251; Anal. Calcd. for $C_{14}H_{13}N_5$ (251.12) (%): C, 66.92; H, 5.21; N, 27.87. Found (%): C, 66.87; H, 5.32; N, 27.92. ^{1}H-NMR of Zn(II) complex (DMSO-d_6): δ 8.2 (d, 1H, pyridine ring), 7.5-7.6 (m, 2H, benzimidazole ring), 7.4 (d, 1H, pyridine ring), 7.1 (m, 2H, benzimidazole ring), 7.0 (s, 2H, N-CH_2-N), 6.7-6.8 (m, 2H, pyridine ring), 5.6 (d, 2H, N-CH_2-NH), 3.8 (s, 1H, NH).

2.6. Compound 3 (L_3): 3-(pyrimidin-2-yl)-1,2,3,4-tetrahydrobenzo[4,5] Imidazo[1,2-a][1,3,5] Triazine

White solid; yield 44%; m.p: 207 °C; IR (KBr): 3396 (NH stretching), 3055 (Aromatic CH_2), 2867 (CH_2 stretching), 1653 (C=N), 1590 (NH bending), 1348s (C-N); ^{1}H-NMR (DMSO-d_6): δ 8.6 (t, 1H, pyrimidine ring), 7.5-7.6 (d, 2H, pyrimidine ring), 7.2-7.3 (t, 2H, benzimidazole ring), 7.1 (d, 2H, benzimidazole ring), 6.8 (d, 2H, N-CH_2-NH), 6.6 (s, 2H, N-CH_2-N), 3.5 (s, 1H, NH); Mass spectrum (ESI) [M]+ = 252; Anal. Calcd. for $C_{13}H_{12}N_6$ (252.27) (%): C, 61.89; H, 4.79; N, 33.31. Found (%): C, 61.82; H, 4.74; N, 33.36. ^{1}H-NMR of Zn(II) complex (DMSO-d_6): δ 8.9 (t, 1H, pyrimidine ring), 7.7-7.8 (d, 2H, pyrimidine ring), 7.5-7.6 (t, 2H, benzimidazole ring), 7.3 (d, 2H, benzimidazole ring), 7.0 (d, 2H, N-CH_2-NH), 6.8 (s, 2H, N-CH_2-N), 3.7 (s, 1H, NH).

2.7. Antibacterial Activity

The in-vitro antibacterial activity of Mannich bases(L_1-L_3) and their metal (II) complexes (C_1-C_{12}) was assayed against two Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) bacterial strains by the reported method. [20,21] The stock solution (1 mg/ml) of the test chemical was prepared by dissolving 10 mg of the test compound in 10 ml of Dimethyl sulfoxide (DMSO) solvent. The stock solution was suitably diluted with sterilized distilled water to

get dilution of 100, 50 and 25 mgml^{-1}. Control for each dilution was prepared by diluting 10 ml of solvent instead of stock solution with sterilized distilled water. The wells (6 mm in diameter) were dug in the agar media with the help of a sterile metallic borer. Two to eight hours old bacterial inocula containing approximately 104-106 colony forming units (CFU/mL) were spread on the surface of the nutrient agar with the help of a sterile cotton swab. The prepared concentrations of the test sample were introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, Gentamycin, served as negative and positive controls, respectively. The plates were incubated immediately at 37°C for 24 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). In order to clarify any effect of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains.

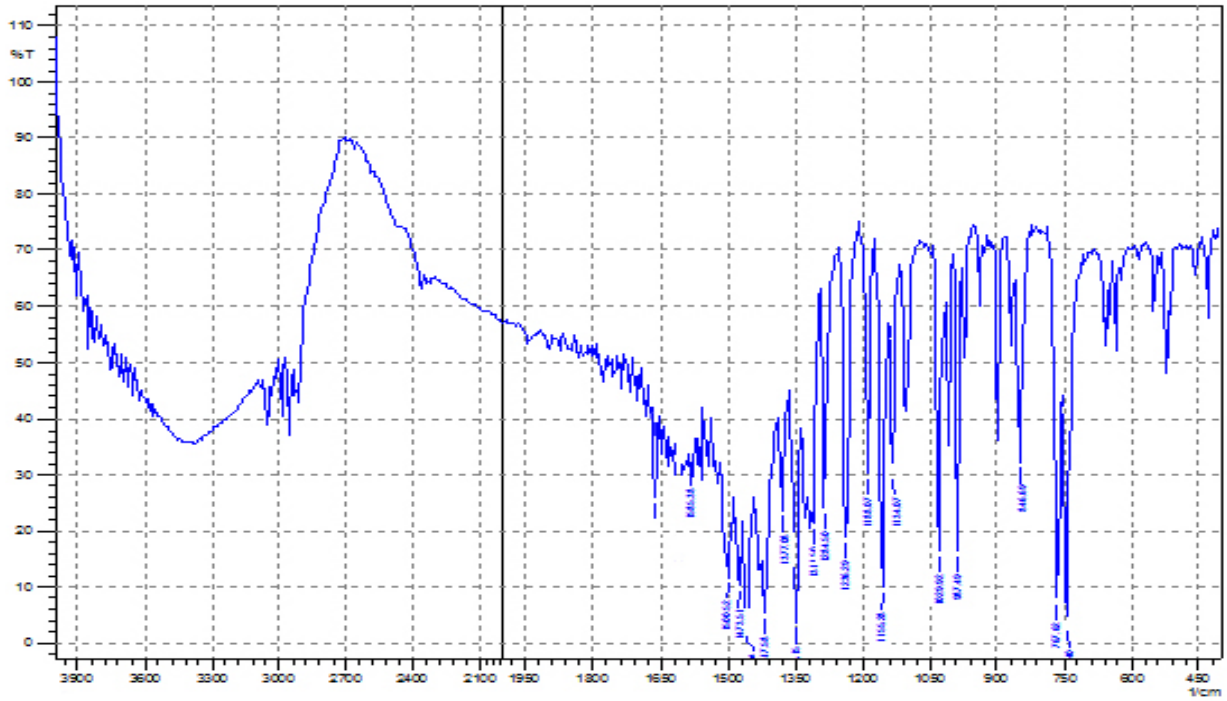


Figure 3. Representative IR spectra of L_2

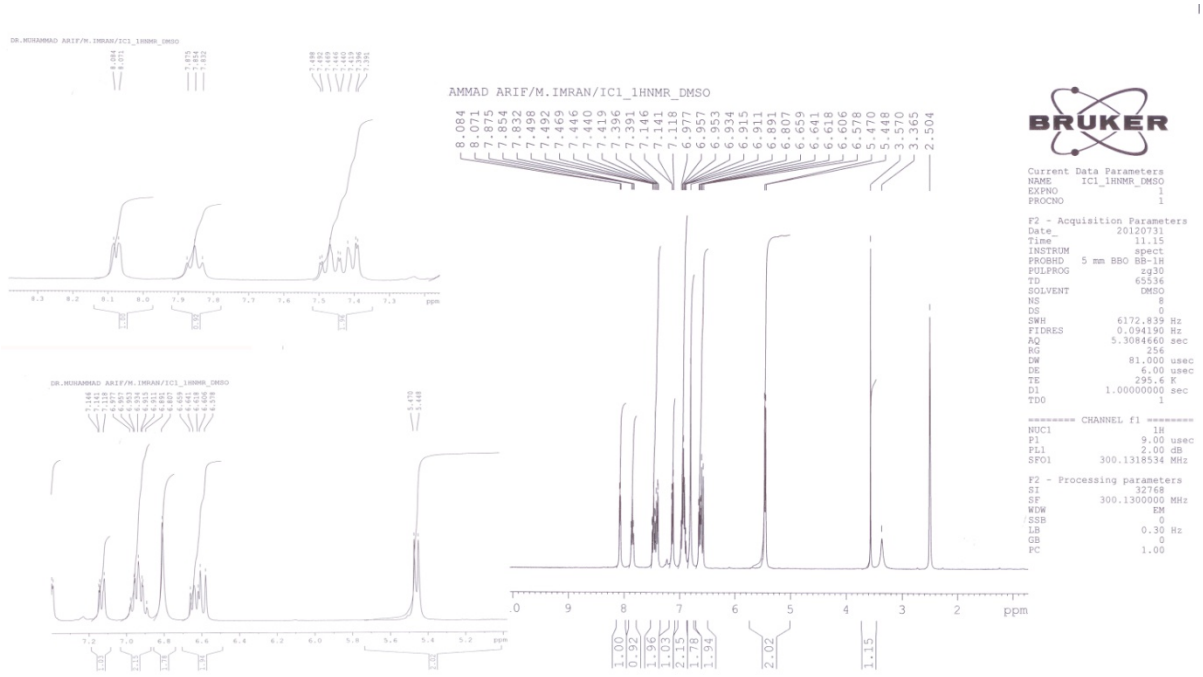


Figure 4. Representative ^{1}NMR spectra of L_2

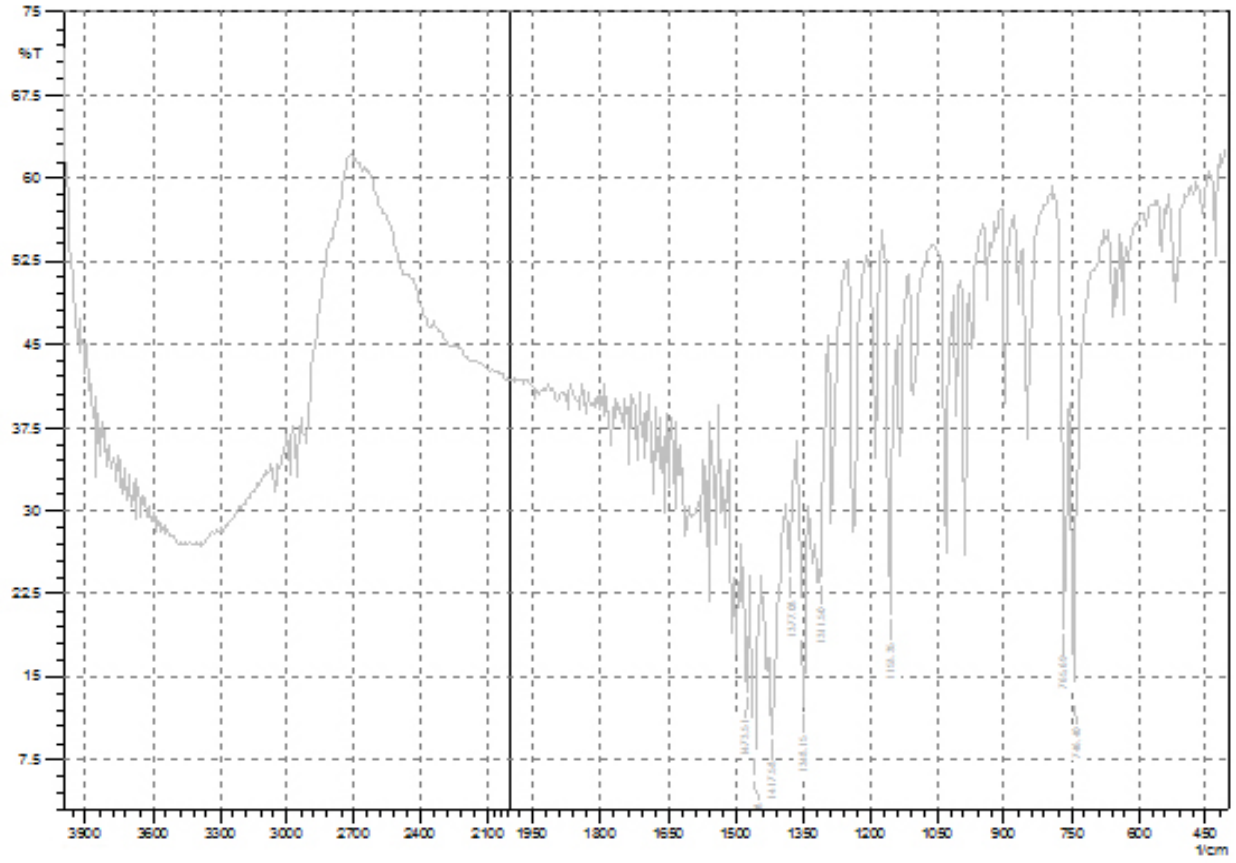


Figure 5. Representative IR spectra of L_3

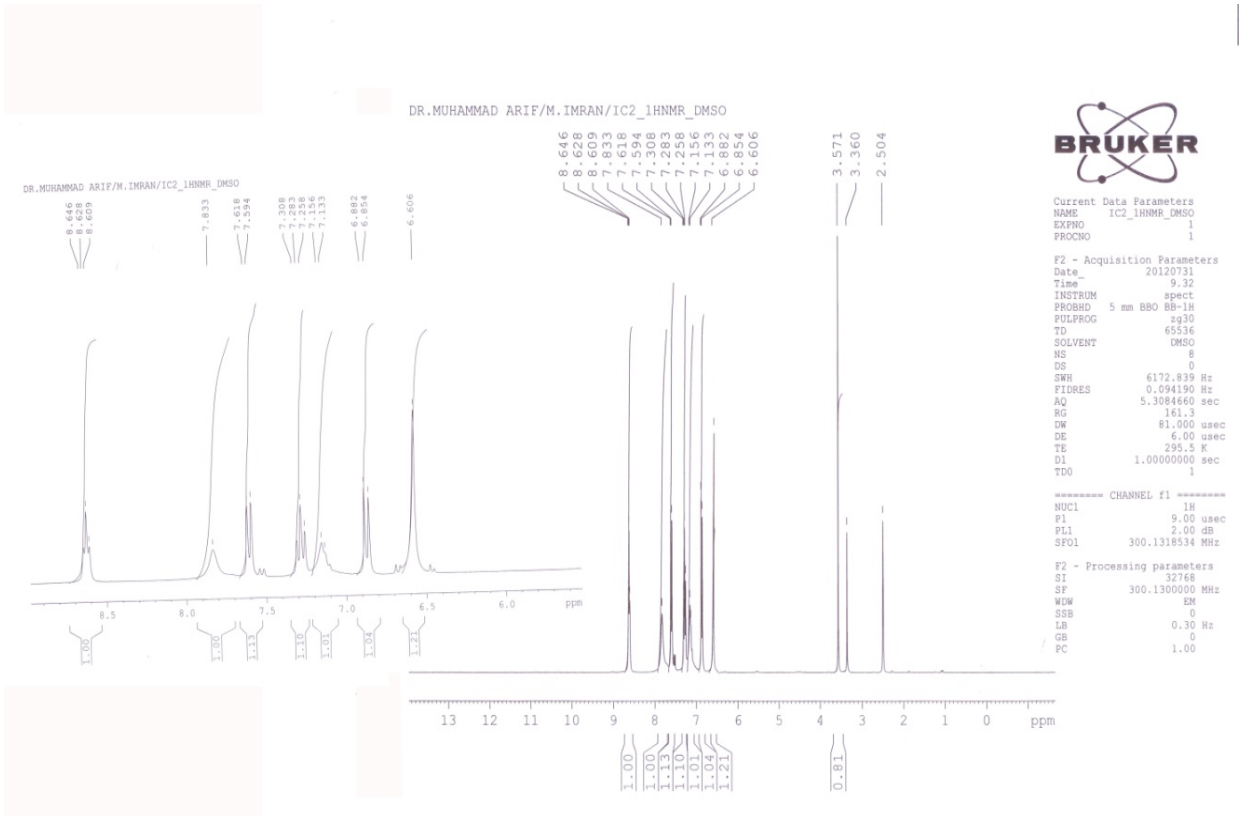


Figure 6. Representative ^{1}NMR spectra of L_3

2.8. Antifungal Activity

All the compounds (L_1-L_3) and their metal(II) complexes (C_1-C_{12}) were studied against five fungal cultures (*Aspergillus niger, Penicillium expansum, Rhizopus nigricans, Trichoderna lignorum, Botrydepladia thiobromine*) for Antifungal activities. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 105 cfu ml-1 fungal spore suspensions and transferred to petri plates. The stock solution of test chemical was prepared and diluted to 100, 50 and 25mgml^{-1}. Discs soaked in 20 ml of prepared concentrations of all compounds were placed at different positions on the agar surface. The plates were incubated at 32 °C. The percentage inhibition was calculated after seven days and compared with standard drugs Fluconazole.[22]

2.9. In vitro Cytotoxicity

The synthesized compounds and their Zn(II), Co(II), Cu(II) and Ni(II) complexes were screened for their cytotoxicity (brine shrimp bioassay) by using the protocol of Meyer et al. [21] Brine shrimp (Artemia salina leach) eggs were hatched in a shallow rectangular plastic dish (22 x 32 cm) filled with artificial seawater, which was prepared with a commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the minor compartment was open to ordinary light. After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 ml of DMSO. From this stock solution 100, 50 and 25 mgml^{-1} were transferred to nine vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 ml of DMSO only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 ml of seawater and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with seawater to 5 ml per vial. After 24 h the number of survivors was counted. Data were analyzed by a Finney computer program to determine the LD_{50} values.[23]

3. Results and Discussion

3.1. IR Spectra

The important IR spectral bands of the Mannich bases and their metal complexes along with their tentative assignments are given in the experimental and Table 1.

The ligands show a broad band at 3382-3396 cm^{-1} and sharp bands at 1653-1660 cm^{-1}, 2847-2867 cm^{-1}, assigned to NH stretching, υ(C=N) and CH_2 stretching vibrations respectively. In the complexes of Ligands 1 and 2, the azomethine frequency shows a downfall (15-30 cm^{-1}) indicating coordination through N atom. This is further supported by the appearance of new bands at 450-486 cm^{-1} due to υ(M-N) bond.[24]

The C-O stretching mode in Ligand 1 is usually found around 1242cm^{-1}. The shift of C-O stretching towards higher frequencies in the metal complexes suggest M-O bond formation and appearance of new bands at 510-540 cm^{-1} support the formation of M-O bond.[25]

The presence of coordinated water molecule in the complex is indicated by the appearance of a broad band at 3226-3460 cm^{-1} and two weak bands in the region 754-784 cm-1 and 700-718 cm^{-1} due to (-OH) rocking and wagging mode of vibrations, respectively.[26]

3.2. ^{1}H NMR Spectra

^{1}H NMR spectra of the free ligands and their diamagnetic zinc(II) complexes were recorded in DMSO-d_6. The ^{1}H NMR spectral data along with the possible assignments is recorded in the Experimental. Mannich bases (L_1-L_3) have shown the peak at 4.3-6.8 ppm due to methylene linkage (2H, -CH_2-) formed between benzimidazole moiety and amino compound. Mannich bases reaction can be further confirmed by the absence of peak for (-NH) secondary amino group of benzimidazole ring system.[27] Two triplet peaks at 4.1 and 2.9 ppm indicated the presence of CH_2 groups in L_1. A multiplet peak at 6.8-6.9 ppm indicated the presence of phenyl group. In L2, peaks at 8.0, 7.1 and 6.5-6.6 ppm indicated the presence of pyridine ring. Multiplet peaks at 7.3-7.4 and 6.9 ppm confirms the presence of benzimidazole moiety. A triplet peak at 8.6 ppm and doublet peak at 7.5-7.6 ppm indicated the presence of pyrimidine ring in L_3. In L_3, presence of benzimidazole group indicated by the appearance of triplet peak at 7.2-7.3 and doublet peak at 7.1 ppm. The ^{1}H NMR spectra of Zn(II) complexes lend further support to the mode of bonding discussed in their IR spectra. The coordination of the nitrogen and oxygen is inferred by the downfield shift (0.3-0.6) of the surrounding proton signals in the complexes. All other protons underwent a downfield shift by 0.2-0.4 ppm due to the increased conjugation[28] and coordination with the metal atom.

3.3. Electronic Spectra and Magnetic Moments

The electronic spectra if Ni(II) complexes exhibits three bands at 9400-9800, 15400-15900 and 24200-24600, which may reasonably be assignable to $^3A_{2g}(F)\rightarrow{}^3T_{2g}(F)$, $^3A_{2g}(F)\rightarrow{}^3T_{1g}(F)$ and $^3A_{2g}(F)\rightarrow{}^3T_{1g}(P)$ transitions, respectively. The magnetic moments for Ni(II) complexes (2.98-3.27 BM) are within the range of an octahedral geometry.[7] The electronic spectra of Co(II) complexes shows absorption bands at 9200-9400, 17700-17900 and 19200-19500 assignable to $^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$, $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ and $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$ transitions, respectively. The magnetic moment values of Co(II) complexes are 4.78-4.89 BM, suggesting an octahedral geometry.[29] The observed magnetic moments for Cu(II) complexes are 1.72-1.83 BM and the band observed at 14500-14900 ($^2E_g\rightarrow{}^2T_{2g}$) in the electronic spectra suggest an octahedral geometry.[30] The Zn(II) complexes are diamagnetic as expected for d_{10} system. (Table 2)

Table 1. The important infrared frequencies (in cm^{-1}) of Zn(II), Co(II), Cu(II) and Ni(II) complexes

Code	Complex	M:L	υ(C=N)	υ(M-N)	υ(M-O)	υ(C-O)
C_1	$[Zn(L_1)_2(OH_2)_2]Cl_2$	1:2	1632s	455m	510m	1340m
C_2	$[Zn(L_2)_2(OH_2)_2]Cl_2$	1:2	1630m	462m	-	-
C_3	$[Zn(L_3)_2(OH_2)_2]Cl_2$	1:2	1634m	458m	-	-
C_4	$[Co(L_1)_2(OH_2)_2]Cl_2$	1:2	1624m	460m	520m	1348m
C_5	$[Co(L_2)_2(OH_2)_2]Cl_2$	1:2	1626s	475m	-	-
C_6	$[Co(L_3)_2(OH_2)_2]Cl_2$	1:2	1622m	465m	-	-
C_7	$[Cu(L_1)_2(OH_2)_2]Cl_2$	1:2	1624s	486m	526m	1349
C_8	$[Cu(L_2)_2(OH_2)_2]Cl_2$	1:2	1620m	483m	-	-
C_9	$[Cu(L_3)_2(OH_2)_2]Cl_2$	1:2	1618m	486m	-	-
C_{10}	$[Ni(L_1)_2(OH_2)_2]Cl_2$	1:2	1620m	482s	524m	1368
C_{11}	$[Ni(L_2)_2(OH_2)_2]Cl_2$	1:2	1632m	478m	-	-
C_{12}	$[Ni(L_3)_2(OH_2)_2]Cl_2$	1:2	1628s	462m	-	-

Table 2. Electronic Spectra and magnetic moments of Mannich base metal(II) complexes

Code	Mag. moments (μ_{eff} in BM)	λ_{max} (cm^{-1} mol^{-1})	Assignment
C_1	Dia	-	-
C_2	Dia	-	-
C_3	Dia	-	-
C_4	4.89	9300 17900 19200	$^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$
C_5	4.78	9400 17700 19400	$^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$
C_6	4.82	9300 17700 19300	$^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$
C_7	1.78	14500	$^2E_g\rightarrow{}^2T_{2g}$
C_8	1.81	14800	$^2E_g\rightarrow{}^2T_{2g}$
C_9	1.83	14700	$^2E_g\rightarrow{}^2T_{2g}$
C_{10}	3.10	9800 15900 24500	$^3A_{2g}(F)\rightarrow{}^3T_{2g}(F)$ $^3A_{2g}(F)\rightarrow{}^3T_{1g}(F)$ $^3A_{2g}(F)\rightarrow{}^3T_{1g}(P)$
C_{11}	2.98	9700 15400 24600	$^3A_{2g}(F)\rightarrow{}^3T_{2g}(F)$ $^3A_{2g}(F)\rightarrow{}^3T_{1g}(F)$ $^3A_{2g}(F)\rightarrow{}^3T_{1g}(P)$
C_{12}	3.14	9400 15600 24400	$^3A_{2g}(F)\rightarrow{}^3T_{2g}(F)$ $^3A_{2g}(F)\rightarrow{}^3T_{1g}(F)$ $^3A_{2g}(F)\rightarrow{}^3T_{1g}(P)$

Table 3. Physical and Analytical data of Zn(II), Co(II), Cu(II) and Ni(II) complexes

Code	Yield (%)	(ESI) [M]+	Molar conductance (Ohm^{-1} cm^{-2} mol^{-1})	Calculated (Found) %			
				C	H	N	M
C_1	74	760	135	53.66 (53.60)	5.30 (5.30)	14.72 (14.27)	8.59 (8.57)
C_2	71	674	142	49.83 (49.81)	4.48 (4.31)	20.75 (20.73)	9.69 (9.77)
C_3	68	676	127	46.14 (46.05)	4.17 (4.19)	24.83 (24.23)	9.66 (9.12)
C_4	76	753	120	54.12 (54.01)	5.34 (5.36)	14.85 (14.45)	7.81 (7.65)
C_5	72	667	129	50.31 (50.37)	4.52 (4.26)	20.95 (20.91)	8.82 (8.97)
C_6	70	669	135	46.58 (46.37)	4.21 (4.26)	25.07 (25.31)	8.79 (8.31)
C_7	75	759	130	53.79 (53.36)	5.31 (5.32)	14.76 (14.62)	8.37 (8.25)
C_8	72	673	122	49.97 (49.94)	4.49 (4.28)	20.81 (20.77)	9.44 (9.53)
C_9	76	675	132	46.26 (46.02)	4.18 (4.18)	24.90 (24.86)	9.41 (9.41)
C_{10}	74	754	136	54.14 (54.08)	5.34 (5.24)	14.85 (14.72)	7.78 (7.63)
C_{11}	76	666	140	50.33 (50.41)	4.53 (4.47)	20.96 (20.89)	8.78 (8.66)
C_{12}	70	668	136	46.60 (46.52)	4.21 (4.22)	25.08 (25.01)	8.76 (8.67)

3.4. Thermogravimetric Analysis

Thermogravimetric analyses (TGA) for the complexes were carried out from room temperature to 700°C. Coordinated waters are usually eliminated at higher temperatures than those of hydration[31,32] usually in the temperature range 100-350°C. The complexes may decompose in more than two steps with the formation of intermediates[33,34] calculated and estimated mass losses are comparable.

The TGA curves of all Mannich-base metal complexes (C_1-C_{12}) have two stages of mass loss, at 102-227°C and at 227-595°C. Weight loss in the range 102-227°C with estimated mass loss of 4.1-4.85% in all the complexes indicates the loss of two coordinated waters. From 227°C to 595°C, a sharp decrease in weight indicated the loss of one Mannich base from the complexes with estimated mass loss of 43.17-44.10% for all the complexes respectively(Table 4).

The molecular masses determined mass spectrometrically (Table 3) also confirmed the ML2 composition. Based upon experimental evidence thus obtained, the complexes were characterized as six coordinates with the two positions occupied by water. The hydrated complexes have significant importance in the enzymatic systems, as the substrates can bind to metal by substituting the coordinated water. The proposed structures of the complexes under investigation, on the basis of above experimental evidence, are shown in Figure 7, 8 and 9. Unsuccessful attempts to isolate crystals suitable for X-ray analysis prevented further structure elucidation.

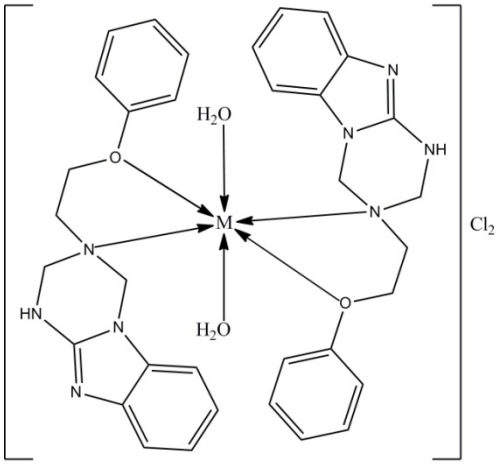


Figure 7. Proposed structure of metal complexes of L_1, where; M=Co(II), Ni(II), Cu(II) and Zn(II)

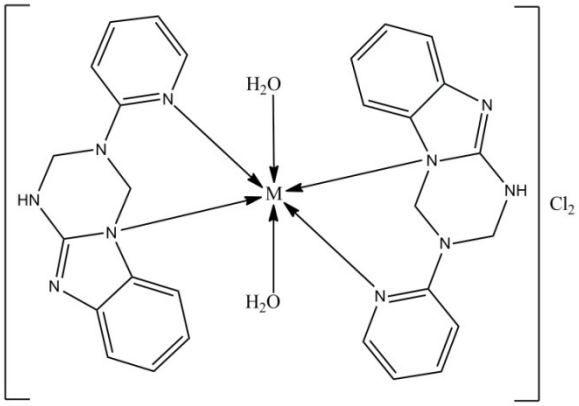


Figure 8. Proposed structure of metal complexes of L_2, where; M=Co(II), Ni(II), Cu(II) and Zn(II)

Table 4. Thermogravimetric analysis (TGA) results of Mannich base metal(II) complexes

Code	Temperature range °C	Mass loss % Found (Calcd)	Assignment
C_1	155-220 229-590	4.64 (4.69) 43.39 (43.45)	Loss of $2H_2O$ Loss of L_1
C_2	150-227 227-584	4.81 (4.92) 43.17 (43.17)	Loss of $2H_2O$ Loss of L_2
C_3	102-198 229-595	4.41 (4.42) 43.73 (43.77)	Loss of $2H_2O$ Loss of L_3
C_4	158-222 232-595	4.68 (4.69) 43.76 (43.77)	Loss of $2H_2O$ Loss of L_1
C_5	145-224 238-589	4.85 (4.86) 43.54 (43.54)	Loss of $2H_2O$ Loss of L_2
C_6	108-203 227-590	4.44 (4.48) 44.08 (44.10)	Loss of $2H_2O$ Loss of L_3
C_7	162-218 227-595	4.66 (4.67) 43.54 (43.58)	Loss of $2H_2O$ Loss of L_1
C_8	157-223 235-574	4.82 (4.84) 43.27 (43.28)	Loss of $2H_2O$ Loss of L_2
C_9	109-212 232-594	4.42 (4.44) 43.88 (43.90)	Loss of $2H_2O$ Loss of L_3
C_{10}	162-227 239-595	4.69 (4.71) 43.77 (43.78)	Loss of $2H_2O$ Loss of L_1
C_{11}	147-215 236-565	4.85 (4.86) 43.56 (43.57)	Loss of $2H_2O$ Loss of L_2
C_{12}	114-211 230-574	4.44 (4.41) 44.10 (44.13)	Loss of $2H_2O$ Loss of L_3

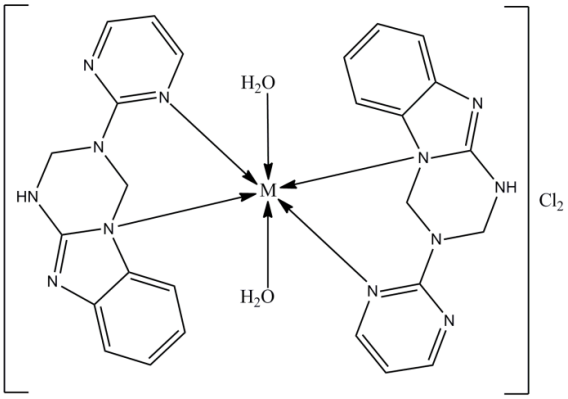


Figure 9. Proposed structure of metal complexes of L_3, where; M=Co(II), Ni(II), Cu(II) and Zn(II)

3.5. Antibacterial Activity

The in vitro antibacterial activity was assayed against two Gram-positive (*Bacillus subtilis Staphylococcus aureus*) and two Gram-negative strains (*Escherichia coli, Pseudomonas aeruginosa*) according to the reported method.[35] Gentamycine was used as a comparative drug. (Figure 10).

Table 5. Antibacterial results of the Mannich bases (L_1-L_3) and their metal(II) complexes (C_1-C_{12})

Code	Conc. (μgml^{-1})	Antibacterial activity (zone of inhibition in %)			
		E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*
L_1	100	73	71	66	51
L_2	100	73	72	69	50
L_3	100	76	77	68	54
C_1	100	70	71	69	51
C_2	100	77	76	68	56
C_3	100	85	80	71	64
C_4	100	76	74	69	57
C_5	100	79	77	71	65
C_6	100	84	80	75	64
C_7	100	70	76	71	60
C_8	100	79	77	72	69
C_9	100	87	82	77	65
C_{10}	100	79	74	73	64
C_{11}	100	81	79	76	70
C_{12}	100	86	83	79	73
Standard	100	100	100	100	100

The antibacterial results suggested that all the Mannich base derivatives of benzimidazole were found to be biologically active. Among the ligands, L_3 displayed the highest rate of suppression. This may be due to the additional nitrogen atom in the ring which increases the bonding with bacterial cell membrane.

In vitro efficiency of all the compounds against Gram-positive bacterial strains was much lower than Gram-negative. *E. coli* was the most susceptible species, affected by all the compounds. The activity against *S. aureus* is only mild even at 100 μgml^{-1} concentration.

It is known[36,37] that chelation tends to make the ligands act as more potent antibacterial agents. It is observed that growth inhibiting activity of metal(II) complexes of Mannich bases is superior when compared with the ligands (L_1-L_3 vs. C_1-C_{12}). (Table 5)

3.6. Antifungal Activity

Antifungal activity was determined in vitro against *Aspergillus niger, Penicillium expansum, Rhizopus nigricans, Trichoderna lignorum* and *Botrydepladia thiobromine*. The inhibition results were compared with the standard drug Fluconazole. (Figure 11)

Mannich bases expressed lower antifungal activity as compared to antibacterial. All the derivatives were efficacious against *A. niger* and *P. expansum*. The results for *R. nigricans* and *T. lignorum* were satisfactory only by a high concentration, showing zone of inhibition in 60-70% range. *B. thiobromine* was almost insusceptible for all the Mannich bases, but showed moderate results for complexes at higher concentration. (Table 6)

Table 6. Antifungal results of the Mannich bases (L_1-L_3) and their metal(II) complexes (C_1-C_{12})

Code	Conc. (μgml^{-1})	Antifungal activity (zone of inhibition in %)				
		A. niger	*P. expansum*	*R. nigricans*	*T. lignorum*	*B. thiobromine*
L_1	100	70	71	63	62	57
L_2	100	73	70	65	63	58
L_3	100	78	77	68	66	61
C_1	100	73	74	69	63	57
C_2	100	77	76	65	65	60
C_3	100	78	76	74	70	6
C_4	100	74	74	64	64	59
C_5	100	76	76	63	65	60
C_6	100	81	80	75	71	64
C_7	100	76	75	66	63	60
C_8	100	77	75	68	66	62
C_9	100	82	82	76	71	66
C_{10}	100	75	74	69	67	58
C_{11}	100	78	79	72	70	60
C_{12}	100	84	83	74	71	63
Standard	100	100	100	100	100	100

3.7. Cytotoxic Bioassay

Table 7. Brine shrim.p bioassay data of the Mannich bases (L_1-L_3) and their metal(II) complexes (C_1-C_{12})

Code	LD_{50} (M)	Code	LD_{50} (M)
C_1	$>3.36 \times 10^{-3}$	C_7	$>1.41 \times 10^{-3}$
C_2	1.03×10^{-4}	C_8	2.70×10^{-4}
C_3	$>2.94 \times 10^{-3}$	C_9	$>1.73 \times 10^{-3}$
C_4	$>1.30 \times 10^{-3}$	C_{10}	1.01×10^{-4}
C_5	3.11×10^{-4}	C_{11}	1.83×10^{-5}
C_6	$>1.31 \times 10^{-3}$	C_{12}	1.67×10^{-4}

Cytotoxicity (brine shrimp bioassay) was determined for all the compounds and their metal(II) complexes. The cytotoxicity is expressed as LD_{50}, i.e. concentration, at which 50% of the viable cells were killed under the assay conditions.

From the data recorded in Table 7, it is evident that only one Mannich base (L_2) displayed potent cytotoxic activity (LD_{50} = 1.03 x 10-4 moles/mL) against Artemia Salina, while the other synthesized compounds were almost inactive in this assay. It was interesting to note that complexation with Nickel increased cytotoxicity, all other metal(II) complexes showed clearly higher values. These findings may help to serve as a basis for future direction towards the development of bacteriostatic agents of lower cytotoxicity (Table 7).

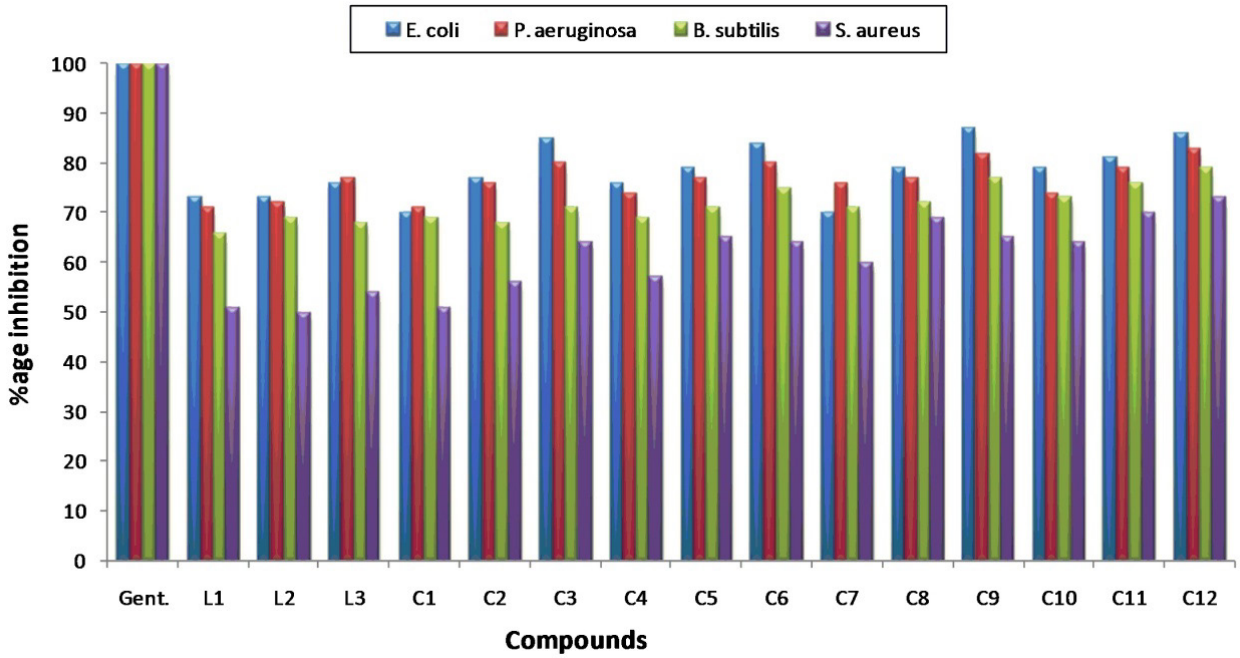


Figure 10. In vitro antibacterial spectrum of Mannich bases (L_1-L_3) and Zn(II), Co(II), Cu(II) and Ni(II) complexes (C_1-C_{12}) and gentamycine (Std.) at 100 µgml-1 concentration

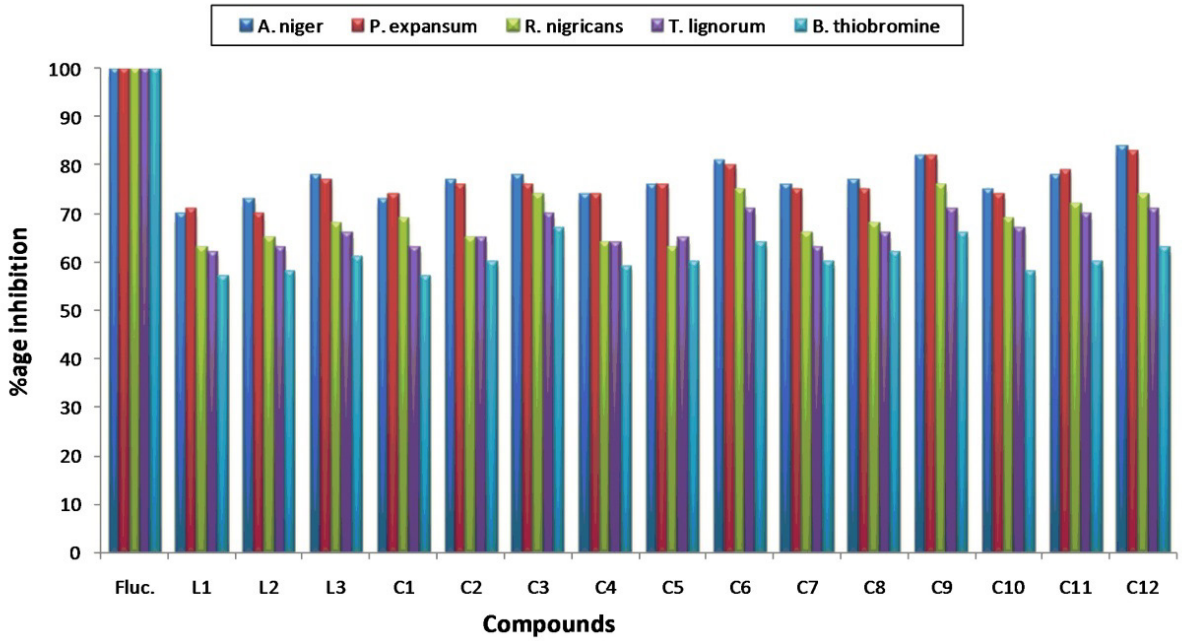


Figure 11. In vitro antifungal spectrum of Mannich bases (L_1-L_3) and Zn(II), Co(II), Cu(II) and Ni(II) complexes (C_1-C_{12}) and Fluconazole (Std.) at 100 µgml-1 concentration

4. Conclusions

The synthesized Mannich bases act as bidentate ligands. The IR, TGA, conductivity, magnetic and electronic studies confirms that the ligands coordinated to metal through oxygen and nitrogen as donor atoms. All the derivatives and their metal(II) complexes were evaluated in vitro against four bacterial (two Gram-negative, two Gram-positive) and five fungal strains. Compounds showed more potency against bacteria. *E. coli, P.* aeruginosa, *A. niger and P. expansum* were the most susceptible species.

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