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Metal-based Antimicrobial agents: Synthesis, Characterization and Biological studies of Mannich base derivatives of Benzimidazole and their Metal complexes

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Abstract: Novel Mannich base derivatives of Benzimidazole were prepared through the condensation reaction of benzimidazole derivative with formaldehyde and primary and/ secondary amine. Zinc(II), copper(II), nickel(II) and cobalt(II) complexes of Mannich bases have also been synthesized. All the compounds were fully characterized by, elemental analyses, magnetic moment determination, molar conductivity measurement, thermogravimetric analysis, spectral and analytical data. Experimental results showed that metal complexes act as bi-dentate ligands towards divalent metal ions via azomethine-N and deprotonated-O while suggesting an octahedral geometry. All the compounds were screened for in-vitro antibacterial and antifungal activity against various bacterial and fungal strains. Almost all the compounds showed good potent activity against microorganisms. It was also seen that compounds with complexed form were more active as compared to un-complexed form. The prepared compounds were also screened for their cytotoxicity and results showed that only Ni(II) complexes exhibit some cytotoxicity while all other compounds were almost inactive.

Keywords: Mannich Bases, Benzimidazole, Metal Complexes, Biological Activity, Cytotoxicity

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] 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, anthelmintic[5], antifungal[6], anti-inflamatory[7], antiviral[8] and analgesic[9] properties. The compounds with azomethine group in its structure are known as Schiff bases, which are synthesized by the condensation reaction of primary amines and active carbonyl groups.[10] Schiff bases of benzimidazole have been reported with remarkable antibacterial[11], antimicrobial[12] and antiproliferative[13] activities. In addition to their biological importance, benzimidazoles form stable complexes with various transition metals.[14] Transition metal complexes of 2-substituted benzimidazole and benzimidazole-based mixed ligands have been reported with mono-, bi- and tri dentate coordination behavior.[15-19]

The worthwhile biological activities of Mannich and Schiff bases have been guiding for the synthesis of novel Mannich and Schiff bases in a single molecule. 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 6 Mannich and Schiff bases and 24 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.

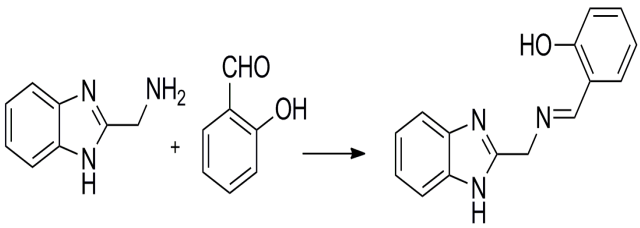
2. Experimental

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 and ^{13}C-NMR spectra of compounds were recorded with a Bruker Spectrospin Avance DPX-400 using TMS as internal standard and d6 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 Schiff base (Scheme 1)

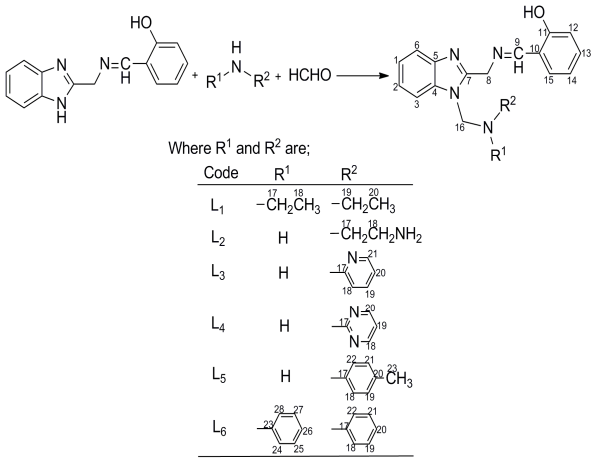
Schiff base of 2-amino(methyl)benzimidazole with salicyldehyde was prepared according to the method reported in literature[20].



Scheme 1. *Synthesis of Schiff base.*

2.3. Synthesis of Mannich bases (Scheme 2)

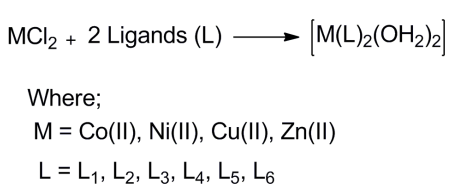
To a solution of synthesized Schiff base (0.05 mole) in 30 ml of ethanol, 0.05 mole formaldehyde and 0.05 mole of respective primary and/ secondary amine were added. The mixture was stirr for 1 h and then allowed to reflux for 7-8 h at 75 °C. A clear solution was obtained. The completion of reaction was monitored by TLC. The reaction mixture was kept at 5 °C for overnight; the precipitates formed were filtered, washed with acetone and dried.



Scheme 2. *Synthesis of Mannich bases.*

2.4. Synthesis of metal complexes (Scheme 3)

To a hot magnetically stirred THF solution of Mannich bases (L_1-L_6) (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.



Scheme 3. *Synthesis of Metal complexes.*

2.5. (E)-2-((((1H-Benzo[d] Imidazol-2-yl) Methyl)Imino) Methyl) Phenol

Yellow solid; yield 70%; m.p: 208-210 °C; IR (KBr): 3474 (-NH Stretching), 3438 (-OH), 3055 (Aromatic -CH_2), 1648 (-C=N), 1544 (-NH Bending), 1346 (C-N), 1236 (C-O, phenolic); ^{1}H-NMR (DMSO-d_6): δ 11.92 (b, 1H, OH), 8.81 (s, 1H, -CH=N), 7.42 (m, 4H), 7.66 (d, *J* = 7.4 Hz, 1H, aromatic), 7.01 (d, *J* = 4.5 Hz, 3H), 9.76 (b, 1H, -NH), 5.09 (s, 2H, -CH_2-); ^{13}C NMR (DMSO-d_6): δ 162.1, 131.6, 124.2, 119.3, 116.5, (C18, C14, C13, C15, C17, Phenol ring), 158.3 (C12, imine, -CH=N), 140.5 (C8, imidazole, N=C-N), 121.6, 109.8, 136.2 (C1, C3, C5, benzimidazole, Phenyl ring), 54.7 (C10, Aliphatic CH_2); Mass spectrum (ESI) $[M]^+$ = 251; Anal. Calcd. for $C_{15}H_{13}N_3O$ (251.28) (%): C, 71.69; H, 5.21; N, 16.72. Found (%): C, 71.60; H, 5.30; N, 16.80.

2.6. (E)-2-((((1-((Diethylamino) Methyl)-1H-Benzo [D]imidazol-2-yl) Methyl)Imino)Methyl) Phenol (L_1)

Dark brown solid; yield 72%; d.p: 220-224 °C; IR (KBr): 3442 (OH), 3055 (Aromatic CH_2), 2954 (CH_3 Stretching), 2847 (CH_2 Stretching), 1655 (C=N), 1355 (C-N, Amine), 1242 (C-O, phenolic); ^{1}H-NMR (DMSO-d_6): δ 12.86 (b,

1H, OH), 8.82 (s, 1H, -CH=N), 7.47 (m, 4H), 7.66 (d, *J* = 7.4 Hz, 1H, aromatic), 7.01 (d, *J* = 4.5 Hz, 3H), 5.05 (s, 2H, $-CH_2-$), 4.92 (s, 2H, $-CH_2-$), 2.32 (m, 4H, CH_2), 1.27 (m, 6H, CH_3); ^{13}C NMR (DMSO-d_6): δ 162.1, 132.6, 124.2, 117.3, 116.5, (C18, C14, C13, C15, C17, Phenol ring), 155.3 (C12, imine, -CH=N), 140.5 (C8, imidazole, N=C-N), 122.6, 109.8, 134.2 (C1, C3, C5, benzimidazole, Phenyl ring), 54.7 (C10, Aliphatic CH_2, -N-CH_2-C), 79.2 (C20, Aliphatic CH_2, -N-CH_2-N), 43.1 (C22, Ethyl CH_2), 17.2 (C25, CH_3); Mass spectrum (ESI) $[M]^+$ = 336; Anal. Calcd. for $C_{20}H_{24}N_4O$ (336.43) (%): C, 71.40; H, 7.19; N, 16.65. Found (%): C, 72.42; H, 7.23; N, 16.67; ^1H-NMR of Zn(II) complex (DMSO-d_6): δ 9.41 (s, 1H, -CH=N), 7.71 (m, 4H), 8.02 (d, *J* = 7.4 Hz, 1H, aromatic), 7.45 (d, *J* = 4.5 Hz, 3H), 5.58 (s, 2H, $-CH_2-$), 5.41 (s, 2H, $-CH_2-$), 2.77 (m, 4H, CH_2), 1.57 (m, 6H, CH_3); ^{13}C NMR of Zn(II) complex (DMSO-d_6): δ 162.9, 133.1, 124.8, 117.7, 117.4, (C18, C14, C13, C15, C17, Phenol ring), 155.7 (C12, imine, -CH=N), 141.3 (C8, imidazole, N=C-N), 123.3, 110.2, 134.7 (C1, C3, C5, benzimidazole, Phenyl ring), 55.3 (C10, Aliphatic CH_2, -N-CH_2-C), 79.7 (C20, Aliphatic CH_2, -N-CH_2-N), 43.9 (C22, Ethyl CH_2), 17.8 (C25, CH_3).

2.7. (E)-2-((((1-(((2-Aminoethyl) Amino) Methyl)-1H-Benzo [D]Imidazol-2-yl) Methyl) Imino) Methyl) Phenol (L_2)

Light yellow solid; yield 68%; d.p: 225-228 °C; IR (KBr): 3433 (OH), 3382 (NH stretching), 3342 (NH_2 Stretching), 3055 (Aromatic CH_2), 2854 (CH_2 Stretching), 1660 (C=N), 1574 (NH bending), 1564 (NH_2 bending), 1348 (C-N, aromatic amine), 1254 (C-O, phenolic); ^1H-NMR (DMSO-d_6): δ 12.92 (b, 1H, OH), 8.81 (s, 1H, -CH=N), 7.38 (m, 4H), 7.62 (d, *J* = 7.4 Hz, 1H, aromatic), 6.98 (d, *J* = 4.5 Hz, 3H), 5.19 (m, 2H, NH_2), 5.02 (s, 2H, $-CH_2-$), 4.92 (d, *J* = 6.2 Hz, 2H, $-CH_2-$), 3.81 (b, 1H, NH), 2.79 (m, 2H, CH_2), 2.57 (m, 2H, $-CH_2-$); ^{13}C NMR (DMSO-d_6): δ 164.4, 131.5, 124.4, 119.2, 116.5, (C18, C14, C13, C15, C17, Phenol ring), 157.5 (C12, imine, -CH=N), 144.2 (C8, imidazole, N=C-N), 122.6, 109.8, 136.2 (C1, C3, C5, benzimidazole, Phenyl ring), 64.1 (C20, Aliphatic CH_2, -N-CH_2-N), 53.4 (C10, Aliphatic CH_2), 42.1 (C22, Aliphatic CH_2, -N-CH_2-C), 37.3 (C24, Aliphatic CH_2, $_2$HN-CH_2-C); Mass spectrum (ESI) $[M]^+$ = 323; Anal. Calcd. for $C_{18}H_{21}N_5O$ (323.39) (%): C, 66.85; H, 6.54; N, 21.65. Found (%): C, 66.87; H, 6.57; N, 21.66; ^1H-NMR of Zn(II) complex (DMSO-d_6): δ 9.38 (s, 1H, -CH=N), 7.79 (m, 4H), 8.02 (d, *J* = 7.4 Hz, 1H, aromatic), 7.48 (d, *J* = 4.5 Hz, 3H), 5.49 (m, 2H, NH_2), 5.33 (s, 2H, $-CH_2-$), 5.21 (d, *J* = 6.2 Hz, 2H, $-CH_2-$), 4.03 (b, 1H, NH), 3.04 (m, 2H, CH_2), 2.98 (m, 2H, $-CH_2-$); ^{13}C NMR of Zn(II) complex (DMSO-d_6): δ 164.9, 132.3, 124.8, 119.6, 116.9, (C18, C14, C13, C15, C17, Phenol ring), 158.2 (C12, imine, -CH=N), 145.1 (C8, imidazole, N=C-N), 123.3, 110.3, 136.9 (C1, C3, C5, benzimidazole, Phenyl ring), 64.8 (C20, Aliphatic CH_2, -N-CH_2-N), 53.6 (C10, Aliphatic CH_2), 42.7 (C22, Aliphatic CH_2, -N-CH_2-C), 37.8 (C24, Aliphatic CH_2, $_2$HN-CH_2-C).

2.8. (E)-2-((((1-((Pyridin-2- Ylamino) Methyl)-1H- Benzo [d] Imidazol-2-yl) Methyl) Imino) Methyl) Phenol (L_3)

Cream color solid; yield 70%; d.p: 237-240 °C; IR (KBr): 3432 (OH), 3396 (NH stretching), 3055 (Aromatic CH_2), 2867 (CH_2 stretching), 1653 (C=N), 1590 (NH bending), 1348s (C-N), 1248 (C-O, phenolic); ^1H-NMR (DMSO-d_6): δ 12.85 (b, 1H, OH), 8.79 (s, 1H, -CH=N), 8.02 (d, 1H, aromatic), 7.52 (m, 3H), 7.42 (m, 4H), 7.66 (d, *J* = 6.4 Hz, 1H, aromatic), 6.97 (d, *J* = 4.5 Hz, 3H), 5.09 (s, 2H, $-CH_2-$), 4.87 (d, *J* = 6.4 Hz, 2H, $-CH_2-$), 3.58 (b, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 161.2, 131.6, 124.2, 119.2, 116.1, (C18, C14, C13, C15, C17, Phenol ring), 156.1 (C12, imine, -CH=N), 141.2 (C8, imidazole, N=C-N), (152.3, 145.7, 138.5, 123.6, 121.3 (C22, C27, C25, C26, C24, 2-Pyridine ring), 121.6, 109.8, 134.2 (C1, C3, C5, benzimidazole, Phenyl ring), 74.2 (C20, Aliphatic CH_2, -N-CH_2-N), 54.7 (C10, Aliphatic CH_2); Mass spectrum (ESI) $[M]^+$ = 357; Anal. Calcd. for $C_{21}H_{19}N_5O$ (357.40) (%): C, 70.57; H, 5.35; N, 19.59. Found (%): C, 70.62; H, 5.41; N, 19.62;); ^1H-NMR of Zn(II) complex (DMSO-d_6): δ 9.40 (s, 1H, -CH=N), 8.32 (d, 1H, aromatic), 7.86 (m, 3H), 7.74 (m, 4H), 8.09 (d, *J* = 6.4 Hz, 1H, aromatic), 7.37 (d, *J* = 4.5 Hz, 3H), 5.39 (s, 2H, $-CH_2-$), 5.19 (d, *J* = 6.4 Hz, 2H, $-CH_2-$), 3.91 (b, 1H, NH); ^{13}C NMR of Zn(II) complex (DMSO-d_6): δ 161.8, 132.1, 124.8, 119.7, 116.4, (C18, C14, C13, C15, C17, Phenol ring), 156.6 (C12, imine, -CH=N), 141.8 (C8, imidazole, N=C-N), 152.8, 146.2, 138.8, 123.9, 121.7 (C22, C27, C25, C26, C24, 2-Pyridine ring), 122.1, 110.3, 134.4 (C1, C3, C5, benzimidazole, Phenyl ring), 74.7 (C20, Aliphatic CH_2, -N-CH_2-N), 55.2 (C10, Aliphatic CH_2).

2.9. (E)-2-((((1-((Pyrimidin-2-Ylamino) Methyl)-1H-Benzo [d]Imidazol-2-yl) Methyl) Imino) Methyl) Phenol (L_4)

White solid; yield 65%; d.p: 238-242 °C; IR (KBr): 3446 (OH), 3396 (NH stretching), 3055 (Aromatic CH_2), 2862 (CH_2 stretching), 1651 (C=N), 1590 (NH bending), 1350 (C-N), 1244 (C-O, phenolic); ^1H-NMR (DMSO-d_6): δ 12.81 (b, 1H, OH), 8.95 (s, 1H, -CH=N), 8.32 (d, *J* = 7.2 Hz, 2H), 7.66 (d, *J* = 6.5 Hz, 1H, aromatic), 7.42 (m, 4H), 7.14 (m, 1H, aromatic), 6.97 (d, *J* = 4.5 Hz, 3H), 5.09 (s, 2H, $-CH_2-$), 4.82 (d, *J* = 6.2 Hz, 2H, $-CH_2-$), 4.15 (b, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 173.5, 154.2, 112.2, (C22, C27, C26, Pyrimidine ring), 162.1, 131.6, 120.3, 119.1, 116.5, (C18, C14, C13, C15, C17, Phenol ring), 158.3 (C12, imine, -CH=N), 140.5 (C8, imidazole, N=C-N), 122.6, 109.8, 136.2 (C1, C3, C5, benzimidazole, Phenyl ring), 63.5 (C20, Aliphatic CH_2, -N-CH_2-N), 54.7 (C10, Aliphatic CH_2); Mass spectrum (ESI) $[M]^+$ = 358; Anal. Calcd. for $C_{20}H_{18}N_6O$ (358.39) (%): C, 67.02; H, 5.06; N, 23.49. Found (%): C, 67.19; H, 5.09; N, 23.53; ^1H-NMR of Zn(II) complex (DMSO-d_6): δ 9.55 (s, 1H, -CH=N), 8.64 (d, *J* = 7.2 Hz, 2H), 7.98 (d, *J* = 6.5 Hz, 1H, aromatic), 7.74 (m, 4H), 7.45 (m, 1H, aromatic), 7.42 (d, *J* = 4.5 Hz, 3H), 5.39 (s, 2H, $-CH_2-$), 5.17 (d, *J* = 6.2 Hz, 2H, $-CH_2-$), 4.55 (b,

1H, NH); ^{13}C NMR of Zn(II) complex (DMSO-d_6): δ 173.9, 154.5, 112.6, (C22, C27, C26, Pyrimidine ring), 162.9, 132.2, 120.7, 119.9, 117.2, (C18, C14, C13, C15, C17, Phenol ring), 158.7 (C12, imine, -CH=N), 141.3 (C8, imidazole, N=C-N), 123.1, 110.2, 136.7 (C1, C3, C5, benzimidazole, Phenyl ring), 64.6 (C20, Aliphatic CH_2, -N-CH_2-N), 55.2 (C10, Aliphatic CH_2).

2.10. (E)-2-((((1-((p-Tolylamino) Methyl)-1H- Benzo[d] Imidazol-2-yl) Methyl) Imino) Methyl) Phenol (L_5)

Off-white solid; yield: 69%; d.p: 240-242 °C; IR (KBr): 3443 (OH), 3392 (NH stretching), 3046 (Aromatic CH_2), 2940 (CH_3 stretching), 2862 (CH_2 stretching), 1656 (C=N), 1586 (NH bending), 1352 (C-N), 1246 (C-O, phenolic); ^{1}H-NMR (DMSO-d_6): δ 12.87 (b, 1H, OH), 8.81 (s, 1H, -CH=N), 7.74 (d, *J* = 5.4 Hz, 1H, aromatic), 7.51 (m, 4H), 7.12 (d, *J* = 8.2 Hz, 2H), 6.87 (d, *J* = 4.5 Hz, 3H), 6.32 (d, 2H), 5.09 (s, 2H, -CH_2-), 4.73 (d, *J* = 6.5 Hz, 2H, -CH_2-), 3.27 (b, 1H, NH), 2.45 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 162.1, 131.6, 122.3, 119.3, 116.5, (C18, C14, C13, C15, C17, Phenol ring), 158.3 (C12, imine, -CH=N), 147.2, 129.2, 127.2, 112.8 (C22, C25, C26, C24, Toluidine ring), 140.5 (C8, imidazole, N=C-N), 121.6, 109.8, 136.2 (C1, C3, C5, benzimidazole, Phenyl ring), 68.2 (C20, Aliphatic CH_2, -N-CH_2-N), 54.7 (C10, Aliphatic CH_2), 27.3 (C29, CH_3); Mass spectrum (ESI) $[M]^+$ = 371; Anal. Calcd. for $C_{23}H_{22}N_4O$ (370.44) (%): C, 74.57; H, 5.98; N, 15.12. Found (%): C, 74.61; H, 6.05; N, 15.17; ^{1}H-NMR of Zn(II) complex (DMSO-d_6): δ 9.41 (s, 1H, -CH=N), 8.09 (d, *J* = 5.4 Hz, 1H, aromatic), 7.83 (m, 4H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.21 (d, *J* = 4.5 Hz, 3H), 6.69 (d, 2H), 5.44 (s, 2H, -CH_2-), 5.09 (d, *J* = 6.5 Hz, 2H, -CH_2-), 3.64 (b, 1H, NH), 2.68 (s, 3H, CH_3); ^{13}C NMR of Zn(II) complex (DMSO-d_6): δ 162.7, 132.4, 122.7, 119.8, 117.2, (C18, C14, C13, C15, C17, Phenol ring), 158.9 (C12, imine, -CH=N), 147.7, 129.8, 128.1, 113.4 (C22, C25, C26, C24, Toluidine ring), 141.2 (C8, imidazole, N=C-N), 122.4, 111.2, 136.9 (C1, C3, C5, benzimidazole, Phenyl ring), 69.5 (C20, Aliphatic CH_2, -N-CH_2-N), 55.6 (C10, Aliphatic CH_2), 27.8 (C29, CH_3).

2.11. (E)-2-((((1-((Diphenylamino) Methyl)-1H-Benzo[d] Imidazol-2-yl) Methyl) Imino) Methyl) Phenol (L_6)

Light skin solid; yield: 64%; d.p: 260-262 °C; IR (KBr): 3440 (OH), 3053 (Aromatic CH_2), 2854 (CH_2 stretching), 1651 (C=N), 1544 (NH bending), 1309 (C-N), 1236 (C-O, phenolic); ^{1}H-NMR (DMSO-d_6): δ 12.90 (b, 1H, OH), 8.74 (s, 1H, -CH=N), 7.59 (d, *J* = 6.5 Hz, 1H, aromatic), 7.42 (m, 4H), 7.01 (d, *J* = 4.5 Hz, 3H), 6.67 (m, 10H, phenyl ring), 5.09 (s, 2H, -CH_2-), 4.87 (s, 2H, -CH_2-), 3.17 (b, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 162.1, 131.6, 124.2, 119.3, 116.5, (C18, C14, C13, C15, C17, Phenol ring), 158.3 (C12, imine, -CH=N), 143.2, 127.3, 120.1, 114.1 (C22, C25, C26, C24, Phenyl ring), 140.5 (C8, imidazole, N=C-N), 121.6, 109.8, 136.2 (C1, C3, C5, benzimidazole, Phenyl ring), 71.3 (C20, Aliphatic CH_2, -N-CH_2-N), 54.7 (C10, Aliphatic CH_2); Mass spectrum (ESI) $[M]^+$ = 433; Anal. Calcd. for $C_{28}H_{24}N_4O$ (432.51)(%): C, 77.75; H, 5.59; N, 12.95. Found (%): C, 77.82; H, 5.60; N, 12.97; ^{1}H-NMR of Zn(II) complex (DMSO-d_6): δ 9.34 (s, 1H, -CH=N), 7.95 (d, *J* = 6.5 Hz, 1H, aromatic), 7.82 (m, 4H), 7.41 (d, *J* = 4.5 Hz, 3H), 6.99 (m, 10H, phenyl ring), 5.41 (s, 2H, -CH_2-), 5.17 (s, 2H, -CH_2-), 3.54 (b, 1H, NH); ^{13}C NMR of Zn(II) complex (DMSO-d_6): δ 162.6, 131.8, 124.6, 119.8, 117.1, (C18, C14, C13, C15, C17, Phenol ring), 159.2 (C12, imine, -CH=N), 143.8, 127.9, 120.7, 114.5 (C22, C25, C26, C24, Phenyl ring), 141.2 (C8, imidazole, N=C-N), 122.3, 110.1, 136.8 (C1, C3, C5, benzimidazole, Phenyl ring), 71.7 (C20, Aliphatic CH_2, -N-CH_2-N), 55.3 (C10, Aliphatic CH_2).

2.12. Antibacterial Activity

The in-vitro antibacterial activity of Mannich bases (**L_1-L_6**) and their metal(II) complexes (**C_1-C_{24}**) was assayed against two Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) bacterial strains by the reported method.[21,22] 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.

2.13. Antifungal Activity

All the compounds (L_1-L_6) and their metal(II) complexes (C_1-C_{24}) 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 25 mg ml^{-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.[23]

2.14. 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.[24] 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 LD50 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.[25]

3. Results and Discussion

3.1. IR Spectra

The important IR spectral bands of the Mannich and Schiff 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 3432-3446 cm^{-1} and sharp bands at 1648-1660 cm^{-1}, 1236-1254 cm^{-1}, assigned to H-bonded –OH, υ(C=N) and phenolic υ(C-O) stretching vibrations respectively. In the complexes, 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.[26]

The shifting of medium to high intensity bands of phenolic C-O bond towards higher frequency (1339-1368 cm^{-1}) and appearance of new bands at 510-540 cm^{-1} support the formation of M-O bond via de-protonation.[27]

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.[28]

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

Code	Complex	υ(C=N)	υ(M-N)	υ(M-O)	υ(C-O)
C_1	$[Zn(L_1)_2(OH_2)_2]$	1632s	455m	510m	1340m
C_2	$[Zn(L_2)_2(OH_2)_2]$	1630m	462m	540m	1349m
C_3	$[Zn(L_3)_2(OH_2)_2]$	1634m	458m	535m	1345m
C_4	$[Zn(L_4)_2(OH_2)_2]$	1628m	460m	512m	1342m
C_5	$[Zn(L_5)_2(OH_2)_2]$	1629m	454m	512m	1347m
C_6	$[Zn(L_6)_2(OH_2)_2]$	1632m	450m	516m	1351s
C_7	$[Co(L_1)_2(OH_2)_2]$	1624m	460m	520m	1348m
C_8	$[Co(L_2)_2(OH_2)_2]$	1626s	475m	514m	1346m
C_9	$[Co(L_3)_2(OH_2)_2]$	1622m	465m	524m	1353m
C_{10}	$[Co(L_4)_2(OH_2)_2]$	1624m	480m	522m	1351m
C_{11}	$[Co(L_5)_2(OH_2)_2]$	1628m	476m	518m	1339m
C_{12}	$[Co(L_6)_2(OH_2)_2]$	1630m	468m	516m	1346m
C_{13}	$[Cu(L_1)_2(OH_2)_2]$	1624s	486m	526m	1349m
C_{14}	$[Cu(L_2)_2(OH_2)_2]$	1620m	483m	522m	1354m
C_{15}	$[Cu(L_3)_2(OH_2)_2]$	1618m	486m	504m	1345m
C_{16}	$[Cu(L_4)_2(OH_2)_2]$	1622m	474m	540m	1349m
C_{17}	$[Cu(L_5)_2(OH_2)_2]$	1625m	472m	510m	1341m
C_{18}	$[Cu(L_6)_2(OH_2)_2]$	1618m	470m	516m	1352m
C_{19}	$[Ni(L_1)_2(OH_2)_2]$	1620m	482s	524m	1368m
C_{20}	$[Ni(L_2)_2(OH_2)_2]$	1632m	478m	529s	1356m
C_{21}	$[Ni(L_3)_2(OH_2)_2]$	1628s	462m	532m	1358m
C_{22}	$[Ni(L_4)_2(OH_2)_2]$	1632m	471m	502m	1342m
C_{23}	$[Ni(L_5)_2(OH_2)_2]$	1630m	485m	515m	1352m
C_{24}	$[Ni(L_6)_2(OH_2)_2]$	1624s	472m	506m	1346m

3.2. ^1H NMR Spectra

^1H NMR spectra of the free ligands and their diamagnetic zinc (II) complexes were recorded in DMSO-d_6. The ^1H NMR spectral data along with the

possible assignments is recorded in the Experimental. The ^{1}H NMR spectra of Schiff base exhibits singlet at 12.92 and 8.81 ppm due to phenolic OH and –CH=N proton respectively.[29] In addition to these signals, Mannich bases (L_1-L_6) have shown the peak at 4.92-4.73 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.[30] A quartet peak at 2.32 and triplet peak at 1.27 ppm indicated the presence of ethyl group in L_1. In L_2, multiplet at 5.19 and 2.57 ppm indicated the presence of –NH_2 and -CH_2 groups respectively. In L_3, presence of pyridine group indicated by the appearance of doublet peak at 8.02 and multiplet at 7.52 ppm. A 2 proton doublet at 8.32 and 1 proton multiplet at 7.14 ppm indicated the presence of pyrimidine group in L_4 Mannich base. In L_5, a singlet peak at 2.45 and doublet peaks at 7.12 and 6.32 ppm indicated the presence of p-toluene group. A multiplet peak at 6.67 ppm indicated the presence of phenyl group in L_6. 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 azomethine nitrogen is inferred by the downfield shift of the -CH=N- proton signal from 8.74-8.95 ppm in the ligands to 9.34-9.55 ppm in the complexes. The hydroxyl proton in the spectra of Zn(II) complexes of ligands disappeared indicating deprotonation and coordination of the oxygen with the metal ion. All other protons underwent a downfield shift by 0.3-0.6 ppm due to the increased conjugation[31] and coordination with the metal atom.

3.3. ^{13}C NMR Spectra

^{13}C NMR spectra of Mannich and Schiff bases were recorded in DMSO and the data are given in the Experimental. The signal due to the azomethine carbon atom appears at δ 153.3-158.3 ppm in all the prepared compounds. Additional signal in the ^{13}C-NMR spectra of the Mannich bases appears at 63.5-79.2 ppm indicating the methylene linkage formed between benzimidazole ring and amino compound.[32]

Table 2. *Electronic Spectral dat and magnetic moments of Mannich base metal(II) complexes*

Code	Mag. moments (μ_{eff} in BM)	λ_{max} (cm^{-1} mol^{-1})	Assignment	Code	Mag. moments (μ_{eff} in BM)	λ_{max} (cm^{-1} mol^{-1})	Assignment
C_1	Dia	-	-	C_{13}	1.78	14500	$^2E_g \rightarrow {}^2T_{2g}$
C_2	Dia	-	-	C_{14}	1.81	14800	$^2E_g \rightarrow {}^2T_{2g}$
C_3	Dia	-	-	C_{15}	1.83	14700	$^2E_g \rightarrow {}^2T_{2g}$
C_4	Dia	-	-	C_{16}	1.77	14800	$^2E_g \rightarrow {}^2T_{2g}$
C_5	Dia	-	-	C_{17}	1.72	14800	$^2E_g \rightarrow {}^2T_{2g}$
C_6	Dia	-	-	C_{18}	1.80	14500	$^2E_g \rightarrow {}^2T_{2g}$
		9300	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$			9800	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
C_7	4.89	17900	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	C_{19}	3.10	15900	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
		19200	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$			24500	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
		9400	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$			9700	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
C_8	4.78	17700	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	C_{20}	2.98	15400	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
		19400	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$			24600	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
		9300	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$			9400	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
C_9	4.82	17700	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	C_{21}	3.14	15600	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
		19300	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$			24400	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
		9200	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$			9700	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
C_{10}	4.86	17900	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	C_{22}	3.21	15400	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
		19500	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$			24200	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
		9300	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$			9600	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
C_{11}	4.84	17700	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	C_{23}	3.27	15600	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
		19500	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$			24400	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
		9200	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$			9500	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
C_{12}	4.88	17800	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	C_{24}	3.16	15700	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
		19400	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$			24500	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$

Table 3. *Thermogravimetric analysis (TGA) results of Mannich base metal(II) complexes.*

Code	Temperature range °C	Mass loss % Found (Calcd)	Assignment	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_{13}	162-218 227-595	4.66 (4.67) 43.54 (43.58)	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_{14}	157-223 235-574	4.82 (4.84) 43.27 (43.28)	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_{15}	109-212 232-594	4.42 (4.44) 43.88 (43.90)	Loss of $2H_2O$ Loss of L_3
C_4	124-223 236-575	4.40 (4.41) 43.75(43.78)	Loss of $2H_2O$ Loss of L_4	C_{16}	128-218 238-584	4.41 (4.42) 43.85 (43.86)	Loss of $2H_2O$ Loss of L_4
C_5	108-200 245-592	4.27 (4.27) 44.04(44.05)	Loss of $2H_2O$ Loss of L_5	C_{17}	112-210 252-594	4.28 (4.29) 44.14 (44.16)	Loss of $2H_2O$ Loss of L_5
C_6	145-225 230-584	3.72 (3.71) 44.80 (44.85)	Loss of $2H_2O$ Loss of L_6	C_{18}	140-227 242-562	3.73 (3.73) 44.89 (44.92)	Loss of $2H_2O$ Loss of L_6
C_7	158-222 232-595	4.68 (4.69) 43.76 (43.77)	Loss of $2H_2O$ Loss of L_1	C_{19}	162-227 239-595	4.69 (4.71) 43.77 (43.78)	Loss of $2H_2O$ Loss of L_1
C_8	145-224 238-589	4.85 (4.86) 43.54 (43.54)	Loss of $2H_2O$ Loss of L_2	C_{20}	147-215 236-565	4.85 (4.86) 43.56 (43.57)	Loss of $2H_2O$ Loss of L_2
C_9	108-203 227-590	4.44 (4.48) 44.08 (44.10)	Loss of $2H_2O$ Loss of L_3	C_{21}	114-211 230-574	4.44 (4.41) 44.10 (44.13)	Loss of $2H_2O$ Loss of L_3
C_{10}	135-220 230-586	4.43 (4.41) 44.10 (44.11)	Loss of $2H_2O$ Loss of L_4	C_{22}	132-227 240-582	4.43 (4.42) 44.11 (44.12)	Loss of $2H_2O$ Loss of L_4
C_{11}	115-210 238-584	4.30 (4.31) 44.38 (44.39)	Loss of $2H_2O$ Loss of L_5	C_{23}	115-221 240-574	4.30 (4.31) 44.40 (44.40)	Loss of $2H_2O$ Loss of L_5
C_{12}	140-220 228-575	3.75 (3.76) 45.10 (45.14)	Loss of $2H_2O$ Loss of L_6	C_{24}	151-217 236-595	3.75 (3.78) 45.11 (45.13)	Loss of $2H_2O$ Loss of L_6

3.4. 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.[33] The electronic spectra of Co(II) complexes shows absorption bands at 9200-9400, 17700-17900 and

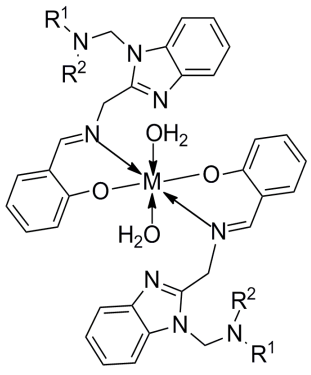
19200-19500 assignable to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions, respectively. The magnetic moment values of Co(II) complexes are 4.78-4.89 BM, suggesting an octahedral geometry.[34] The observed magnetic 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.[35] The Zn(II) complexes are diamagnetic as expected for d^{10} system. (Table 2)

3.5. 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[36,37] usually in the temperature range 100–350 °C. The complexes may decompose in more than two steps with the formation of intermediates[38,39] calculated and estimated mass losses are comparable.

The TGA curves of all Mannich-base metal complexes (C_1-C_{24}) 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 3.72–4.85% 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 43.17–45.11% for all the complexes, respectively. The data are given in Table 3.

The molecular masses determined mass spectrometrically also confirmed the ML_2 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 1. Unsuccessful attempts to isolate crystals suitable for X-ray analysis prevented further structure elucidation.



Where;

M = Co(II), Ni(II), Cu(II), Zn(II)

Figure 1. *Proposed structure of Metal complexes.*

3.6. 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.[22] Gentamycine was used as a comparative drug.

The antibacterial results suggested that all the Mannich and Schiff base derivatives of benzimidazole were found to be biologically active. Aromatic amines seem to be more beneficial than aliphatic amines; L_3, L_4, L_5 and L_6 displayed the highest rate of suppression. Mannich bases L_3 and L_4 expressed almost the same activity as L_4 has one additional Nitrogen atom in the ring, but L_6 was described being the most antibacterial active molecule in this series.

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[40,41] that chelation tends to make the Schiff bases act as more potent antibacterial agents. It is observed that growth inhibiting activity of metal(II) complexes of Mannich and Schiff bases is superior when compared with the ligands (L_1-L_6 vs. C_1-C_{24}).

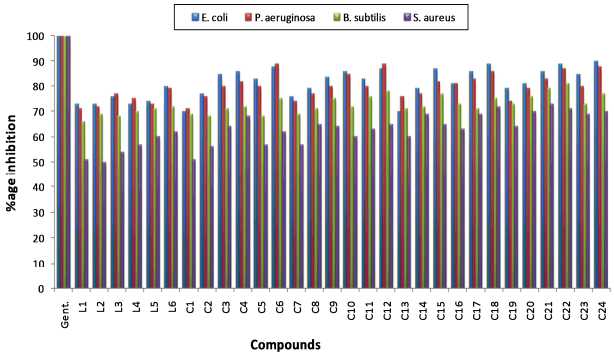


Figure 2. *In vitro antibacterial spectrum of Mannich bases (L_1-L_6) and Zn(II), Co(II), Cu(II), Ni(II) complexes (C_1-C_{24}) and Gentamycine (Std.) at 100 μgml^{-1} concentration.*

3.7. 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.

Mannich and Schiff 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 and Schiff bases, but showed moderate results for complexes at higher concentration.

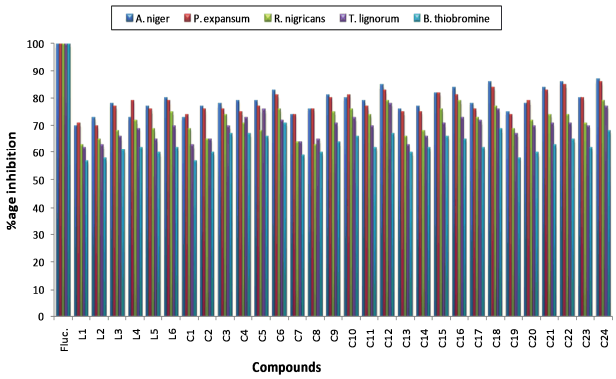


Figure 3. *In vitro antifungal spectrum of Mannich bases (L_1-L_6) and Zn(II), Co(II), Cu(II), Ni(II) complexes (C_1-C_{24}) and Fluconazole (Std.) at 100 μgml^{-1} concentration.*

3.8. Cytotoxic Bioassay

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 5, it is evident that only one Mannich and Schiff 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 copper increased cytotoxicity (L_2 vs. C_{14}, all Cu(II) complexes), all other metal(II) complexes showed clearly higher values (LD_{50} > 1.13 x 10^{-3} moles/mL). These findings may help to serve as a basis for future direction towards the development of bacteriostatic agents of lower cytotoxicity.

Table 4. *Physical and Analytical data of Zn(II), Co(II), Cu(II) and Ni(II) complexes*

Code	Complex (formula weight)	Yield (%)	Molar conductance (Ohm^{-1} cm^{-2} mol^{-1})	Calculated (Found) %			
				C	H	N	M
C_1	$[Zn(L_1)_2(OH_2)_2]$ (774.26)	72	13.1	62.05 (62.06)	6.76 (6.81)	14.47 (14.52)	8.44 (8.47)
C_2	$[Zn(L_2)_2(OH_2)_2]$ (748.18)	71	14.2	57.79 (57.81)	6.19 (6.21)	18.72 (18.73)	8.74 (8.77)
C_3	$[Zn(L_3)_2(OH_2)_2]$ (816.20)	68	10.7	61.80 (61.85)	5.18 (5.19)	17.16 (17.23)	8.01 (8.12)
C_4	$[Zn(L_4)_2(OH_2)_2]$ (818.18)	72	9.6	58.71 (58.77)	4.92 (4.96)	20.54 (20.58)	7.99 (8.05)
C_5	$[Zn(L_5)_2(OH_2)_2]$ (842.28)	70	11.5	65.59 (65.62)	5.74 (5.79)	13.30 (13.31)	7.76 (7.81)
C_6	$[Zn(L_6)_2(OH_2)_2]$ (966.42)	72	8.6	69.63 (69.68)	5.42 (5.43)	11.59 (11.62)	6.76 (6.79)
C_7	$[Co(L_1)_2(OH_2)_2]$ (767.79)	76	7.8	65.57 (65.59)	6.82 (6.83)	14.59 (14.61)	7.67 (7.79)
C_8	$[Co(L_2)_2(OH_2)_2]$ (741.71)	72	6.9	58.29 (58.37)	6.25 (6.26)	18.88 (18.91)	7.94 (7.97)
C_9	$[Co(L_3)_2(OH_2)_2]$ (809.73)	70	5.4	62.29 (62.37)	5.22 (5.26)	17.29 (17.31)	7.27 (7.31)
C_{10}	$[Co(L_4)_2(OH_2)_2]$ (811.71)	73	6.1	59.18 (59.19)	4.96 (4.99)	20.70 (20.71)	7.25 (7.27)
C_{11}	$[Co(L_5)_2(OH_2)_2]$ (835.81)	71	9.3	66.10 (66.17)	5.78 (5.78)	13.40 (13.41)	7.05 (7.11)
C_{12}	$[Co(L_6)_2(OH_2)_2]$ (959.95)	69	4.9	70.06 (70.10)	5.46 (5.49)	11.67 (11.73)	6.13 (6.19)
C_{13}	$[Cu(L_1)_2(OH_2)_2]$ (771.54)	75	7.2	62.26 (62.31)	6.79 (6.81)	14.52 (14.63)	8.23 (8.27)
C_{14}	$[Cu(L_2)_2(OH_2)_2]$ (746.32)	72	7.9	57.93 (57.94)	6.21 (6.28)	18.76 (18.77)	8.51 (8.53)
C_{15}	$[Cu(L_3)_2(OH_2)_2]$ (813.54)	76	4.3	62.00 (62.02)	5.20 (5.28)	17.21 (17.26)	7.81 (7.81)
C_{16}	$[Cu(L_4)_2(OH_2)_2]$ (816.32)	68	11.4	58.85 (58.87)	4.93 (4.97)	20.59 (20.65)	7.78 (7.79)
C_{17}	$[Cu(L_5)_2(OH_2)_2]$ (840.42)	72	12.6	65.74 (65.81)	5.75 (5.81)	13.33 (13.47)	7.56 (7.59)
C_{18}	$[Cu(L_6)_2(OH_2)_2]$ (964.54)	71	8.7	69.73 (69.81)	5.43 (5.47)	11.61 (11.63)	6.58 (6.62)
C_{19}	$[Ni(L_1)_2(OH_2)_2]$ (767.55)	74	13.6	62.59 (62.61)	6.82 (6.83)	14.59 (14.63)	7.64 (7.71)
C_{20}	$[Ni(L_2)_2(OH_2)_2]$ (741.47)	76	7.9	58.34 (58.41)	6.25 (6.37)	18.89 (18.89)	7.91 (7.96)
C_{21}	$[Ni(L_3)_2(OH_2)_2]$ (809.49)	70	12.6	62.31 (62.32)	5.22 (5.22)	17.30 (17.31)	7.25 (7.27)
C_{22}	$[Ni(L_4)_2(OH_2)_2]$ (811.47)	72	11.3	59.20 (59.22)	4.96 (4.99)	20.71 (20.73)	7.23 (7.27)
C_{23}	$[Ni(L_5)_2(OH_2)_2]$ (835.54)	72	9.7	66.12 (66.16)	5.79 (5.79)	13.41 (13.42)	7.02 (7.02)
C_{24}	$[Ni(L_6)_2(OH_2)_2]$ (959.71)	69	12.5	70.08 (70.08)	5.46 (5.47)	11.67 (11.68)	6.11 (6.12)

Table 5. *Brine shrimp bioassay data of the Mannich bases (L_1-L_6) and their metal(II) complexes (C_1-C_{24})*

Code	LD_{50} (M)	Code	LD_{50} (M)	Code	LD_{50} (M)
L_1	$>3.36 \times 10^{-3}$	C_5	$>1.59 \times 10^{-3}$	C_{15}	1.67×10^{-4}
L_2	1.03×10^{-4}	C_6	$>1.44 \times 10^{-3}$	C_{16}	2.87×10^{-4}
L_3	$>2.94 \times 10^{-3}$	C_7	$>1.13 \times 10^{-3}$	C_{17}	1.89×10^{-4}
L_4	$>2.87 \times 10^{-3}$	C_8	$>1.27 \times 10^{-3}$	C_{18}	3.74×10^{-4}
L_5	$>2.61 \times 10^{-3}$	C_9	$>1.69 \times 10^{-3}$	C_{19}	$>1.41 \times 10^{-3}$
L_6	$>2.02 \times 10^{-3}$	C_{10}	$>1.65 \times 10^{-3}$	C_{20}	2.70×10^{-4}
C_1	$>1.30 \times 10^{-3}$	C_{11}	$>1.87 \times 10^{-3}$	C_{21}	$>1.73 \times 10^{-3}$
C_2	3.11×10^{-4}	C_{12}	$>1.63 \times 10^{-3}$	C_{22}	$>1.82 \times 10^{-3}$
C_3	$>1.31 \times 10^{-3}$	C_{13}	1.01×10^{-4}	C_{23}	$>1.61 \times 10^{-3}$
C_4	$>1.82 \times 10^{-3}$	C_{14}	1.83×10^{-5}	C_{24}	$>1.97 \times 10^{-3}$

4. Conclusion

The synthesized Mannich and Schiff bases act as bidentate ligands. The IR, TGA, conductivity, magnetic and electronic studies confirm that the metals are coordinated to azomethine nitrogen and phenolic oxygen. 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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