IN-VITRO ANTIMICROBIAL SCREENING AND COORDINATION BEHAVIOR OF METALS BASED BIDENTATE COMPOUNDS

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ABSTRACT

By condensing ethylene-1,2-diamine with different aldehydes such as benzaldehyde, 4-chloroacetophenone and 2-chlorobenzaldeyhde within 1:2 molar ratio, resulted new series of Schiff base ligands **(L^1)-(L^3)** containing bidentate nitrogen atom. Their metal complexes were synthesized by coordinating the ligands with transition metals as Co(II), Cu(II), Ni(II) and Zn(II) and exhibited octahedral geometry. Their characterization was done with the help of spectral, physical and analytical analysis. Spectral and elemental analysis of all bidentate ligands and their corresponding 3*d*-metal chelates was consistent with their proposed structures, signifying the high purity of these compounds. For *in-vitro* studies, these metal complexes along their ligands were screened against the six bacterial strains; *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus* and *Streptococcus faecalis*. Six fungal strains; *Aspergillus niger, Trichophyton mentogrophytes, Epidermophyton floccosum, Trichophyton schoenleinii, Microscopum canis* and *Fusarium culmorum* were used to study antifungal activity of the compounds. Bioactivity results exhibited that metal complexes showed higher antimicrobial potential as compared with their corresponding ligands. The enhanced activity resulted due to chelation that decreases the polarity of metal ions by complexing with bidentate ligands.

Keywords: Bidentate Schiff bases; Metal(II) Complexes; Antibacterial; Antifungal; MIC Activity.

INTRODUCTION

Biologically active metal complexes and their ligands have always been the subject of much research. Due to the extensive pharmacological properties, they have been used in the last few years [1]. Biological activity of these compounds is considered due to the potential sites of Schiff bases. Bidentate, tridentate and tetradentate Schiff bases along with their metal complexes consisting of donor oxygen and nitrogen atoms play significant roles towards potent biological potentials [2]. In inorganic chemistry, Schiff bases have a special importance because with most transition metals they can easily form stable complexes [3,4]. The importance of Schiff base metal complexes evidenced by the developments and approaches in bioinorganic chemistry. These metal complexes were proved useful for many biologically important species [5]. The compounds were tested for the antibacterial [6], anticancer [7], antifungal [8] and antioxidant [9] activities due to the increased demand of Schiff base derived metal complexes.

Many Schiff bases had been synthesized and still today, many metal complexes are being synthesized due to their increasing activities. In pharmacological point of view, Schiff bases and their metal complexes have great interest, which contain nitrogen and oxygen donor atoms [10]. Those compounds were for their widespread pharmacological properties since many years. It is an important and well known reality that chelation/coordination occurs between the metals and active sites of the nitrogen and oxygen containing ligands [11]. In the last few years, those coordinated compounds have achieved more attention which contain biologically active ligands [12]. It has been proved that the cause and cure of many diseases is chelation. Many metal complexes of Schiff bases have been examined for their biological activities like antimicrobial [13], DNA interaction [14], anticarcinogenic [15], herbicidal [16], antineoplastic [17], COX-inhibitor [18], antiepileptic [19], antiviral [20], antioxidant [21] and analgesic [22]. However, important biological properties like antipathogenic [23], anticancer [24], fungicidal [25] and antidiabetic [26] are more pronounced for transition metal chelates of ethylene-1,2-diamine.

Due to the important structural properties, biological activities and applications of compounds containing ethylene-1,2-diamine, we wish to synthesize new Schiff base ligands **(L^1)-(L^3)** along with their transition metals complexes including Co(II), Cu(II), Ni(II) and Zn(II) **(1)-(12)**. These compounds were examined for the antifungal and antibacterial activity by using different bacterial and fungal strains. The results supported the fact that these newly synthesized compounds have shown good antimicrobial activities.

EXPERIMENTAL

General

The chemicals purchased from Sigma Aldrich were of analytical grade and no further purification was required before use. Methanol used in all the synthetic reactions of ligands and their complexes was purified before use by using authentic method from literature. In all experiments, de-ionized and redistilled water was used. Gallenkamp apparatus employed for determining the melting points and decomposition temperatures of synthesized ligands and their respective metal complexes, respectively. Nicolet FT-IR Impact 400D infrared spectrophotometer utilized for IR spectra using KBr matrix within 3700-370 cm^{-1} region. A Bruker Avance 300 MHz instrument was used to record the ^1H and ^{13}CNMR spectra of the compounds. The mass spectrometric analysis was done by Agilent Technologies Time-of-Flight 6210 LC/MS. A Hitachi UV-3200 spectrophotometer was used for UV spectra. The CHN Analyzer on Perkin Elmer 2400 series II, employed for prepared compounds elemental analysis (C, H and N %). Inolab Cond 720 Conductivity Bridge was used for the measurement of the molar conductivity of transition metal complexes using 10^{-3} M solutions in DMF solvent at 25 °C. By using Hg-acetate ligand as a standard and Stanton SM12/S Gouy balance, the magnetic moments of the metal(II) chelates were determined at 25 °C temperature.

Synthetic Chemistry of Ligands (L^1)-(L^3)

Synthesis of the ligand, *N,N'-bis[phenylmethylidene]ethane-1,2-diamine* was done by the following process in which solution of ethylene-1,2-diamine (0.67 mL, 10 mmol) was refluxed in methanol then 20 mL methanolic solution of benzaldehyde (2.04 mL, 20 mmol) was added in 1:2 molar ratio. After 30 minutes, addition of few drops of H_3PO_4 followed by 6 hours reaction mixture refluxing. The reaction progress was supervised through thin layer

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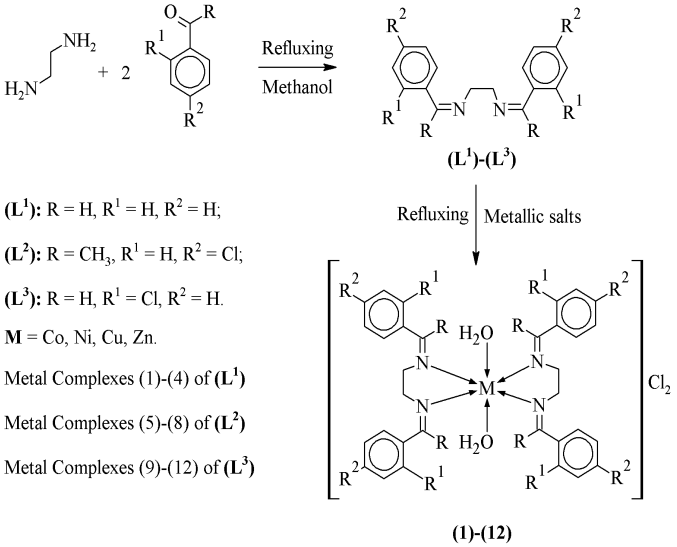
chromatography (TLC). After termination of starting material, the reaction mixture cooled upto 25 °C. Then, the solvent was evaporated by using the rotary evaporator. Then, the obtained product was thoroughly washed by MeOH and air dried. Re-crystallization was done in hot ether/MeOH (1:2). The other two ligands **(L^2)-(L^3)** were synthesized with same procedure.



Scheme 1. Synthetic Scheme of Ethylene-1,2-diamine Schiff Base Ligands **(L^1)-(L^3) a**nd their Divalent Metal Chelates **(1)-(12)**

N,N'-bis[phenylmethylidene]ethane-1,2-diamine (L^1)

Yield (1.85g, 78 %), mp 127 °C; color (light yellow). IR (KBr, cm^{-1}): 1638 (HC=N), 1531, 1490 (C=C); NMR-^{1}H (d_6-DMSO; ppm): 3.81 (s, 4H), 7.22-7.75 (m, 10H), 8.75 (s, HC=N); ^{13}C (d_6-DMSO: ppm): 58.8, 128.7, 129.9, 131.5, 138.8, 161.7; MSESI: $[M]^+$ = 236.31. Anal. calcd. for $C_{16}H_{16}N_2$ (236.31): C, 81.32; N, 11.85; H, 6.82. Found: C, 81.27; N, 11.82; H, 6.79.

N,N'-bis[1-(4-chlorophenyl)ethylidene]ethane-1,2-diamine (L^2)

Yield (2.35 g, 71 %); mp 165 °C; color (yellow). IR (KBr, cm^{-1}): 1635 (C=N), 1539, 1497 (C=C); NMR-^{1}H (d_6-DMSO; ppm): 2.18 (s, 6H), 3.85 (s, 4H), 7.30 (dd, 4H, J = *7.9* Hz), 7.64 (dd, 4H, J = *7.9* Hz); ^{13}C (d_6-DMSO: ppm): 18.2, 60.1, 129.7, 130.9, 136.1, 138.8, 163.2; MSESI: $[M]^+$ = 333.25. Anal. calcd. for $C_{18}H_{18}Cl_2N_2$ (333.25): C, 64.87; Cl, 21.28; N, 8.41; H, 5.44. Found: C, 64.81; Cl, 21.22; N, 8.35; H, 5.41.

N,N'-bis[(2-chlorophenyl)methylidene]ethane-1,2-diamine (L^3)

Yield (2.25g, 74 %); mp: 174 °C, color (dark yellow). IR (KBr, cm^{-1}): 1638 (HC=N), 1536, 1494 (C=C). NMR-^{1}H (d_6-DMSO; ppm): 3.87 (s, 4H), 6.87 (t, 2H, J = *8.4* Hz), 6.95 (d, 2H, J = *7.8* Hz), 7.32 (t, 2H, J = *7.9* Hz), 7.59 (d, 2H, J = *7.9* Hz), 8.95 (s, HC=N). ^{13}C (d_6-DMSO: ppm): 58.1, 127.6, 129.6, 130.7, 132.3, 134.1, 139.5, 163.5. MSESI: $[M]^+$ = 305.20. Anal. calcd. for $C_{16}H_{14}Cl_2N_2$ (305.20): C, 62.97; H, 4.62; N, Cl, 23.23; 9.18. Found: C, 62.92; H, 4.58; Cl, 23.17; N, 9.15.

Synthetic Chemistry of Divalent Transition Metal Complexes (1)-(12)

By using the following method, all the complexes were synthesized. The Schiff base ligand (10 mmol) in MeOH (30 mL) was refluxed. Then metal (II) chloride.nH_2O (5 mmol) in methanolic solution (20 mL) was added (n = 0, 2 or 6). For refluxing the reaction mixture for 3 hours, formed precipitates were cooled at 25 °C overnight followed by filtration, washing with methanol/diethyl ether. The obtained product filtered precipitates were dried, recrystallized in a mixture of hot MeOH/ether (1:2) to get pure product.

NMR Spectral Data of the Divalent Zinc Complexes

[Zn(L^1)$_2$Cl$_2$] (4) NMR-^{1}H (d_6-DMSO; ppm): 10.5 (s, 4H, H_2O), 3.90 (s, 4H), 7.30-7.87 (m, 10H), 8.86 (s, HC=N). ^{13}C (d_6-DMSO: ppm): 162.6, 139.6, 59.5, 129.4, 130.7, 131.9.

[Zn(L^2)$_2$Cl$_2$] (8) NMR-^{1}H (d_6-DMSO; ppm): 10.5 (s, 4H, H_2O), 2.25 (s, 6H), 3.94 (s, 4H), 7.38 (dd, 4H, J = *7.9* Hz), 7.74 (dd, 4H, J = *7.9* Hz). ^{13}C (d_6-DMSO: ppm): 163.9, 139.6, 18.7, 60.9, 130.4, 131.5, 136.8.

[Zn(L^3)$_2$Cl$_2$] (12) NMR-^{1}H (d_6-DMSO; ppm): 10.5 (s, 4H, H_2O), 3.98 (s, 4H), 6.95 (t, 2H), 7.02 (d, 2H, J = *8.4* Hz), 7.39 (t, 2H, J = *7.8* Hz), 7.65 (d, 2H, J = *7.9* Hz), 9.08 (s, HC=N). ^{13}C (d_6-DMSO: ppm): 163.7, 140.9, 17.9, 19.7, 21.5, 58.8, 127.5, 128.4, 131.7, 133.3, 136.9.

***In-vitro* Antibacterial Activity**

The *in-vitro* antibacterial action of all the prepared Schiff base ligands **(L^1)-(L^3)** and their corresponding divalent transition metal chelates **(1)-(12)** was examined against bacterial strains (*E. coli, S. aureus, K. pneumoniae, S. faecalis, B. subtilis* and *P. aeruginosa*) using agar well method [27] as depicted in **Table 3**. Incubation of the test organism with 10 mL of nutrient broth was performed at 37°C for 24 hours. To 60 mL of molten agar, 0.6 mL of broth culture of the tested organism was added with the help of sterile pipette. Then it was cooled upto 45°C, mixed properly and poured within sterile Petri dishes. After some time when the agar was set and turned to hard material, the needed number of holes were cut with cork borer. After this, agar plugs were separated. For different organisms, different cork borers were used. The test sample (100 µL) was dissolved within DMSO, transferred to suitably marked cups with the help of a 0.1 mL pipette. The same concentration was used for the typical antibacterial drug, streptomycin (1 mg/mL) within same solvent. To allow diffusion of sample, the plates were kept for 2 hours at 25 °C followed by 24 hours incubation at 37 °C. The diameters (mm) of the inhibition zones were calculated.

***In-vitro* Antifungal Activity**

Six fungal species, *Trichophyton schoenleinii, Fusarium culmorum, Microscopum canis, Aspergillus niger, Epidermophyton floccosum* and *Trichophyton mentogrophytes* were used to study the antifungal activity according to the reported procedure [28] as recorded in **Table 4**. Stock solution was made by liquefying the test sample within DMSO. By properly mixing the Sabouraud 4 % glucose agar and agar agar, Sabouraud dextrose agar was prepared in distilled water. With the help of magnetic stirrer, it was stirred properly and some amount was distributed into test tubes containing screw caps, autoclaved for 15 min at 121°C. The test tubes were cooled to 50°C. Then, the required concentration of the test samples was poured from a stock solution into the non-solidified Sabouraud agar media by using pipette. Tubes were saved in standing position for solidification at 25 °C. After a week old culture of fungi, 4 mm diameter piece of inoculum obtained, poured into each test tube and incubated.

Minimum Inhibitory Concentration (MIC)

For minimum inhibitory concentration (MIC) studies, those molecules were used which exhibited significant antibacterial activity [29]. The disc diffusion technique was used to study the minimum inhibitory concentration. Discs were prepared containing the sample compounds and standards in the same amount 10, 25, 50 and 100 µgmL^{-1} concentrations.

RESULTS AND DISCUSSION

Three Schiff base ligands **(L^1)-(L^3)** were synthesized by condensing the ethylene-1,2-diamine with benzaldehyde, 4-chloroacetophenone and 2-chlorobenzaldeyhde in molar ratio 1:2, correspondingly (**Scheme 1**). The ligands were stable to air and moisture and they all were colored microcrystal solid compounds. They were melted at 127-174 °C. At room temperature these were will soluble with DMF and DMSO but soluble within MeOH and EtOH upon heating. Ligands were bidentate and they enthusiastically complexed with hydrated metal chlorides of cobalt, nickel, copper and anhydrous zinc chloride to form hydrated metal(II) chealtes in methanol. Strong colors were shown by metal(II) complexes except Zn(II) complexes **(4), (8) & (12)** which were colorless. All the synthesized metal chelates were microcrystalline. These metal complexes decomposed on heating instead of melting. All the synthesized metal complexes were soluble in DMSO, DMF but insoluble in dichloromethane, methanol, ethanol and acetone. The ligands, their metal complexes purity were certified with elemental analysis and spectral data of the compounds. The metal:ligand (1:2) stoichiometry was confirmed by analytical data as depicted in **Table 1.**

Table 1. Physical Measurements and Analytical Data of Metal(II) Complexes **(1)-(12)**.

No	Structure	Yield (%)	MW (g/mol) Formula	M.P (ºC)	Elemental Analysis (%) Calculated (Found)			
					C	H	N	M
(1)	$[Co(L^1)_2(H_2O)_2]Cl_2$	71	[638.49] $C_{32}H_{36}Cl_2N_4O_2Co$	217-218	60.20 (60.12)	5.68 (5.59)	8.77 (8.65)	09.23 (09.14)
(2)	$[Ni(L^1)_2(H_2O)_2]Cl_2$	70	[638.25] $C_{32}H_{36}Cl_2N_4O_2Ni$	237-238	60.22 (60.10)	5.69 (5.58)	8.78 (8.69)	09.20 (09.11)
(3)	$[Cu(L^1)_2(H_2O)_2]Cl_2$	62	[643.10] $C_{32}H_{36}Cl_2N_4O_2Cu$	214-216	59.76 (59.65)	5.64 (5.57)	8.71 (8.66)	09.88 (09.76)
(4)	$[Zn(L^1)_2(H_2O)_2]Cl_2$	66	[644.94] $C_{32}H_{36}Cl_2N_4O_2Zn$	245-247	59.59 (59.47)	5.63 (5.59)	8.69 (8.61)	10.14 (10.05)
(5)	$[Co(L^2)_2(H_2O)_2]Cl_2$	75	[832.38] $C_{36}H_{40}N_4Cl_6O_2Co$	238-239	51.95 (51.88)	4.84 (4.76)	6.73 (6.65)	7.08 (6.97)
(6)	$[Ni(L^2)_2(H_2O)_2]Cl_2$	70	[832.14] $C_{36}H_{40}N_4Cl_6O_2Ni$	261-262	51.96 (51.88)	4.85 (4.76)	6.73 (6.68)	7.05 (6.96)
(7)	$[Cu(L^2)_2(H_2O)_2]Cl_2$	67	[836.99] $C_{36}H_{40}N_4Cl_6O_2Cu$	239-241	51.66 (51.54)	4.82 (4.69)	6.69 (6.56)	7.59 (7.47)
(8)	$[Zn(L^2)_2(H_2O)_2]Cl_2$	73	[838.83] $C_{36}H_{40}N_4Cl_6O_2Zn$	259-261	51.55 (51.49)	4.81 (4.72)	6.68 (6.59)	7.79 (7.65)
(9)	$[Co(L^3)_2(H_2O)_2]Cl_2$	68	[776.27] $C_{32}H_{32}N_4Cl_6O_2Co$	231-232	49.51 (49.43)	4.15 (4.06)	7.22 (7.11)	7.59 (7.48)
(10)	$[Ni(L^3)_2(H_2O)_2]Cl_2$	69	[776.03] $C_{32}H_{32}N_4Cl_6O_2Ni$	256-258	49.53 (49.42)	4.16 (4.05)	7.22 (7.13)	7.56 (7.47)
(11)	$[Cu(L^3)_2(H_2O)_2]Cl_2$	67	[780.89] $C_{32}H_{32}N_4Cl_6O_2Cu$	239-241	49.22 (49.13)	4.13 (4.05)	7.17 (7.06)	8.14 (8.06)
(12)	$[Zn(L^3)_2(H_2O)_2]Cl_2$	72	[782.72] $C_{32}H_{32}N_4Cl_6O_2Zn$	245-246	49.10 (49.03)	4.12 (4.03)	7.16 (7.05)	8.35 (8.23)

IR Spectra

Some unique obtained IR peaks described within experimental section and depicted in **Table 2**. Schiff bases **(L^1)-(L^3)** displayed a distinctive methylimine (C=N) band at 1635-1638 cm^{-1} [29], confirming the condensation product. Due to the presence of C-Cl vibrations, the ligands **(L^2)** and **(L^3)** displayed a band at 815 cm^{-1} [30]. Schiff bases were primarily having two coordination sides with the metal(II) ions evidenced by Schiff base ligands **(L^1)-(L^3)** and their corresponding metal(II) chelates IR spectral bands. The presence of azomethine group was verified by the spectra. The azomethine nitrogen [31] was in coordination with the metal(II) atoms that appeared in the IR bands of divalent metal complexes which moved to 11-20 cm^{-1} lower frequency at 1618-1624 cm^{-1}. The C-Cl vibrations at 815 $cm^{-1}$of the ligands **(L^2)** and **(L^3)** remained unchanged in metal complexes and indicated that they were not involved in the coordination.

The following data proved the chelation process:

- Synthesis of metal complexes was established with new bands existence in the range 534-549 cm^{-1} owing to ν(M-N) vibrations [32] in the IR spectra of metal complexes which were not present in the uncomplexed ligands.
- The broad peaks appeared in the range 3456-3470 cm^{-1} were allotted to H_2O in all studied complexes [11].

These new IR bands were detected only within complex spectra and were not observed in the Schiff bases ligands. Thus, spectral evidences reinforced contribution of nitrogen of azomethine in the coordination. All these facts evidenced the complexation of metal(II) ions with the synthesized Schiff bases.

Table 2. Conductivity, Magnetic and Spectral Data of Metal(II) Complexes **(1)-(12)**.

No	Ω_M ($\Omega^{-1}cm^2mol^{-1}$)	B.M μ_{eff}	λ_m (cm^{-1})	IR (cm^{-1})
(1)	94.8	4.55	8655, 17696 and 29814	3465 (H_2O), 1622 (HC=N), 549 (M-N)
(2)	95.3	3.62	8683, 17717, 25817 and 29781	3455 (H_2O), 1621 (HC=N), 538 (M-N)
(3)	94.8	1.66	8697, 17623 and 29781	3458 (H_2O), 1623 (HC=N), 541 (M-N)
(4)	95.1	Dia	28815	3463 (H_2O), 1621 (HC=N), 535 (M-N)
(5)	95.4	4.38	8686, 17739 and 29841	3460 (H_2O), 1618 (C=N), 541 (M-N)
(6)	95.1	3.44	8728, 17761, 25778 and 29738	3456 (H_2O), 1618 (C=N), 536 (M-N)
(7)	95.3	1.64	8737, 17672 and 29818	3465 (H_2O), 1620 (C=N), 547 (M-N)
(8)	95.5	Dia	28781	3466 (H_2O), 1621 (C=N), 535 (M-N)
(9)	95.2	4.31	8682, 17723 and 29811	3470 (H_2O), 1620 (HC=N), 534 (M-N)
(10)	95.5	3.34	8762, 17712, 25719 and 29813	3466 (H_2O), 1624 (HC=N), 546 (M-N)
(11)	95.7	1.63	8723, 17657 and 29808	3461 (H_2O), 1620 (HC=N), 547 (M-N)
(12)	95.1	Dia	28811	3459 (H_2O), 1621 (HC=N), 539 (M-N)

^{1}H NMR Spectra

All the NMR (^{1}H) spectra of diamagnetic Zn(II) complexes along with Schiff bases were documented in deuterated DMSO and are given within experimental part. NMR (^{1}H) spectrum of the Schiff base ligand **(L^1)** exhibited (C***H*$_2$**) protons of ethylene group and protons of azomethine linkage appeared as a singlet at 3.81 and 8.75, correspondingly. All the aromatic (H) were displayed within 7.22-7.75 (ppm) range as a multiplet. The (C***H*$_2$**) protons of ethylene group and methyl group protons of azomethine linkage of ligand **(L^2)** were appeared as a singlet at 2.18 and 3.85, individually. The aromatic (H) were observed within 7.30-7.64 range as a double doublet (dd). Ligand **(L^3)** exhibited (C***H*$_2$**) protons of ethylene group and protons of methylenimine linkage appeared as a singlet at 3.87 and 8.95, separately.

The protons on R^2 and adjacent aromatic ring carbons were found in the 6.87-7.32 range in the form of triplet (t). The remaining protons of the ring were appeared as a doublet at 6.95-7.59. In the Zn(II) complexes, **(4), (8) & (12)**, proton signals downfield shifting confirmed the coordination of the azomethine nitrogen with zinc metal ion. The downfield shifting of proton signals was because of the discharging of an electronic cloud from ligand donor atoms to the zinc metal ion. All the Zn(II) complexes exhibited a singlet peak at 10.50 because of the presence of H_2O molecules that confirmed the complexation of the H_2O with the zinc metal ion. All the other protons were shifted 0.05–0.15 downfield because of the enhanced conjugation on the coordination of ligands with the zinc metal ion.

Consequently, the number of -H calculated from the integration curves [33] and the found values of the expected elemental analysis were in good agreement with each other.

^{13}C NMR Spectra

DMSO-d_6 was used to record the ^{13}C NMR spectra of diamagnetic Zn(II) complexes along with Schiff base ligands and they are stated in the experimental part. The distinctive azomethine (C=N) carbons were appeared at 161.7-163.5 ppm in NMR (^{13}C) spectra of the Schiff bases **(L^1)-(L^3)**. In azomethine linkage, the carbons (CH_3) were detected, and (CH_2) carbons of ethylene group appeared at 18.3 and (C/CH) carbons of aromatic rings were detected at 58.1-60.1 and 127.3-163.5 ppm, respectively. Downfield shifting was observed in the free ligands from 161.7–163.5 ppm and in their Zn(II) complexes from 162.6–163.9 ppm of the azomethine carbons. It was the electronic density which was shifted towards Zn(II).

Likewise, all carbons of aromatic ring and methyl groups, which were close to the coordinating positions, exhibited another downfield shifted value at 0.5-1.0 ppm. It caused an increase in conjugation and synchronization with the metal(II) atoms. The downfield shifting of all signals proved complexation of the ligands with the zinc metal atoms. Furthermore, involvement of many other carbons strengthen the coordination of metal complexes with the predictable values [34]. In addition, the conclusions drawn from these findings confirmed the binding patterns argued in IR and NMR spectra.

Mass Spectra

The bulk fragmentation mode of ligands **(L^1)-(L^3)** involved the cleavage pattern of bonds as C = N bonds (outer ring), C-Cl and C-CH_3. In the experimental section, the mass spectral data of the ligands with the most stable fragmentation values was displayed. The significant molecular ion peaks were shown by all the ligands. Schiff base data displayed by mass spectrometry intensely exhibited ligands formation having the proposed structure and their binding patterns.

Molar Conductances and Magnetic Measurements

Dimethylformamide was used to investigate the metal(II) complexes molar conductance. Their electrolytic [35] performance were evidenced by the molar conductances data (94.8-95.7 $ohm^{-1}cm^2mol^{-1}$) for the metal(II) complexes **(1-12).** The magnetic moment (B.M) values for all metal complexes **(1-12)** were determined at 25°C. The magnetic moments of Co(II) complexes were detected in the range 4.31–4.55 B.M. The values of molar conductance and magnetic moment are displayed in **Table 2.** It indicated that Co(II) complexes had high-spin, having three unpaired electrons within their octahedral geometry [36]. The magnetic moment values of Ni(II) complexes displayed in between 3.34 and 3.62 B.M. It exhibited the existence of two unpaired electrons for each Ni(II) ion suggesting octahedral [37] geometry of these complexes. For the Cu(II) complex, the measured magnetic moment values is between 1.63 and 1.66 B. M indicating the d^9-system with an unpaired electron signifying octahedral geometry [38]. It is practically observed that all Zn(II) complexes are diamagnetic [39].

Electronic Spectra

The electron spectrum of the Co(II) complexes demonstrated [40] three absorption bands at 8655-8682, 17696-17761 and 29811-29841 cm^{-1} range, which can be attributed to transitions $^4T_{1g} \rightarrow {}^4T_{2g}(F)$, $^4T_{1g} \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g} \rightarrow {}^4Tg(P)$, respectively representing the octahedral geometry for all the Co(II) complexes. The Ni(II) complexes electronic spectral data showed the bands 8683-8762, 17712-17761 and 25719-25817 cm^{-1} [37] which were attributed to the d-d transitions of $^3A_{2g}$ (F) $\rightarrow$ $^3T_{2g}$ (F) and $^3A_{2g}$ (F) $\rightarrow$ $^3T_{1g}$ (F), correspondingly. Because of the transfer of charge from the ligand to the metal, a strong band was observed at 29738-29813 cm^{-1}. The electron spectrum of all complexes of Cu(II) presented absorption bands [41] 8697-8637 and 17623-17672 cm^{-1} order, that may be given along transition $^2E_g \rightarrow {}^2T_{2g}$. The band of high energy at 29781-29818 cm^{-1} was because of the prohibition of ligand→metal charge transfer (CT) process. Based upon the electron spectrum, the octahedral geometry was recommended for Cu(II) complexes. In contrast, the Zn(II) complexes displayed no d-d transition and therefore had diamagnetic nature instead only a strong band of high energy was observed at 28781-28811 cm^{-1} due to CT [42].

Antibacterial Bioassay *(In-vitro)*

The synthesized Schiff base ligands **(L^1)-(L^3)** and their respective transition metal(II) complexes **(1)-(12)** were screened for *in-vitro* antibacterial activities against *E. coli*, *S. aureus, B. subtilis, S. faecalis*, *K. pneumoniae* and *P. aeruginosa* bacterial species as per standard method [27] and the outcomes were stated in **Table 3** and their comparison was presented in **Fig. 1**. The results obtained were compared to the result of the standard streptomycin drug. The synthesized ligand **(L^1)** revealed significant activity (16-19 mm) against *S. faecalis*, *B. subtilis, S. aureus* and *E. coli* bacterial strains and moderate activity (12-14 mm) against *P. aeruginosa* and *K. pneumoniae*. The ligand **(L^2)** have displayed 16 mm activity against *S. faecalis* strain and (12-15 mm) inhibition against other bacterial strains. Likewise, ligand **(L^3)** showed pronounced antibacterial activity (16-19 mm) against all strains with the exception of *P. aeruginosa* strain with moderate (14 mm) activity.

Apart from this, all the metal complexes **(1)-(12)** had a remarkable antibacterial activity (16-26 mm) for all the bacterial strains with the exception of *E. coli* for which the compound **(5)** displayed moderate inhibition (14 mm). The stated data evidently revealed that **(L^3)** exhibited overall significant bacterial activity as a whole when compared to other two ligands. Zn(II) complex **(12)** of ligand **(L^3)** was proved as the most active complex. The metal(II) complexes exhibited higher activity instead of their uncomplexed Schiff bases. Against various bacterial Gram-ive and Gram-+ive strains, all Schiff bases exhibited lesser to moderate antibacterial activity while metal(II) complexes possessed moderate to significant antibacterial activity. It could be seen from the results that the antibacterial activity was improved after the complexation. These consequences further approved our own results and the struggles of some other researchers that those compounds, which are biologically non-active, have become biologically active. Furthermore, biologically active became more active upon complexation/chelation with metal ions [31]. The results showed that upon coordination Schiff bases become powerful antibacterial agents and prevent the development of bacteria more than the corresponding Schiff bases.

Table 3. Antibacterial Bioassay of Ligands **(L^1)-(L^3)** and their Metal(II) Complexes **(1)-(12)**

Antibacterial Activity, Zone of Inhibition (mm)								
Compounds	**(a)**	**(b)**	**(c)**	**(d)**	**(e)**	**(f)**	**(SA)**	**Average**
(L^1)	16	19	14	12	17	16	2.42	15.7
(L^2)	12	16	12	13	15	14	1.63	13.7
(L^3)	17	16	14	18	19	18	1.79	17.0
(1)	25	22	22	19	23	18	2.59	21.5
(2)	24	21	23	17	24	22	2.64	21.8
(3)	19	23	16	22	19	19	2.50	19.7
(4)	20	20	17	16	21	21	2.14	19.2
(5)	14	21	16	17	17	17	3.02	17.0
(6)	20	19	23	19	20	23	1.87	20.7
(7)	16	22	21	18	22	24	2.95	20.5
(8)	18	17	18	16	18	20	1.33	17.8
(9)	20	19	16	22	19	18	2.00	19.0
(10)	18	19	17	17	22	19	1.87	18.6
(11)	19	21	24	20	16	17	2.89	19.5
(12)	24	18	19	26	22	18	3.38	21.2
SD	30	27	28	29	29	28	1.05	28.5

Average Activity of Ligands **(L^1)-(L^3)** = 15.47 mm; Average Activity of Complexes **(1)-(12)** = 19.71 mm; **(a)** *E. Coli* **(b)** *S. Faecalis* **(c)** *P. Aeruginosa* **(d)** *K. Pneumoniae* **(e)** *S. Aureus* **(f)** *B. Subtilis*; **SD**: Standard Drug (Streptomycin); weaker activtiy = 0-10 mm, moderate activtiy = 11-15 mm, above 15 mm = significant activtiy, **SA** = Statistical Analysis.

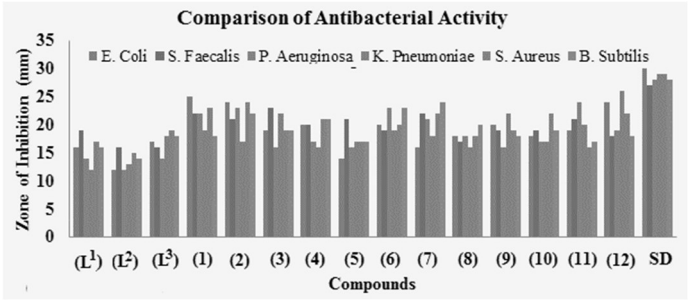


Figure 1. Comparison of Antibacterial Activity of Schiff Bases versus Metal(II) Complexes

Antifungal Bioassay *(In-vitro)*

According to the protocol of the literature [28], the study of antifungal activity of all compounds was done against *Epidermophyton floccosum, Microscopum canis, Trichophyton mentogrophytes, Fusarium culmorum, Aspergillus niger* and *Trichophyton schoenleinii* fungal strains. The inhibition results were compared with the standard drugs, amphotericin B and miconazole **(Table 4)** as shown in **Fig. 2**. The ligand, **(L^1)** exhibited significant (54-57 %) activity against *Trichophyton schoenleinii* and *Epidermophyton floccosum* and a moderate (43-50 %) activity against *Fusarium culmorum, Microscopum canis* and *Trichophyton mentogrophytes*, but no activity was detected against *Aspergillus niger*. On the other side, **(L^2)** have shown significant (56 %) activity against *Epidermophyton floccosum*, while it was moderately active (41-47 %) against *Trichophyton mentogrophytes, Trichophyton schoenleinii, Microscopum canis* and *Aspergillus niger*. Similarly, **(L^3)** presented significant activity (55 %) against *Trichophyton mentogrophytes*, and showed moderate activity (38-46 %) against *Aspergillus niger*, *Epidermophyton floccosum*, *Fusarium culmorum, Microscopum canis* and displayed low (29 %) activity against *Trichophyton schoenleinii*.

The metal(II) complexes performed better and were more active antifungal agents than their non-complexed Schiff bases because of complexation. Compound **(1)** was found to have significant activity (56-73 %) against *Fusarium culmorum, Aspergillus niger, Trichophyton schoenleinii* but *Trichophyton mentogrophytes* and *Epidermophyton floccosum* showed lower activity (21- 27 %). Compound **(2)** was moderately active (35-45%) against *Fusarium oxysporum*, *Aspergillus niger* and all the remaining strains showed, it exhibited low activity (20-27 %). A significant (54-69 %) activity was exhibited by compound **(3)** against fungal strains *Aspergillus niger, Trichophyton mentogrophytes*, and *Trichophyton schoenleinii*, showed moderate activity (43-45 %) against *Fusarium culmorum, Epidermophyton floccosum,* strains while weaker activity (25 %) was displayed for *Microscopum canis*. Similarly, compound **(4)** displayed significant activity (57-75 %) against *Trichophyton schoenleinii*, *Aspergillus niger*, and *Trichophyton mentogrophytes*, medium activity (52 %) against *Microscopum canis* while weaker (17-26 %) activity was presented for *Fusarium culmorum* and *Epidermophyton floccosum*. A significantly strong (57-67 %) activity against *Trichophyton schoenleinii* and *Microscopum canis*, moderate activity (38-43 %) against *Epidermophyton floccosum, Trichophyton mentogrophytes*, *Aspergillus niger* and a very low activity (25 %) against *Fusarium culmorum*, was observed by the compound **(5)**.

The compound **(6)** presented remarkable (66-78 %) activity against *Microscopum canis*, *Trichophyton mentogrophytes*, and *Trichophyton schoenleinii*, moderate (39 %) activity against *Epidermophyton floccosum*, and weaker activity (27-29 %) was displayed for *Fusarium culmorum* and *Aspergillus niger*. Significant activity (56-77 %) was shown for *Aspergillus niger, Epidermophyton floccosum, Fusarium culmorum, Microscopum canis* fungal strains by the compound **(7)**, but moderate (44-49 %) activity was observed against *Trichophyton mentogrophytes* and *Trichophyton schoenleinii* strains. Compound **(8)** exhibited significant activity (68 %) against *Aspergillus niger* fungal strain, compound **(9)** showed activity (61 %) against *Microscopum canis*. Likewise, compound **(10)** showed pronounced activity against *Aspergillus niger* while the compound **(11)** presented (65 %) activity against *Trichophyton schoenleinii* fungal strain. Furthermore, compound **(12)** presented strong activity (70 %) against *Trichophyton mentogrophytes*. All the synthesized metal(II) complexes and Schiff bases displayed weaker to strong antifungal activity against different strains of fungi [33]. It is evident from the data stated in **Table 4** that as compared to other two ligands, **(L^1)** showed comparatively good fungal activity. But the most active complex was Cu(II) complex **(7)** of ligand **(L^2)**. Due to complexation, these newly synthesized divalent metal complexes showed pronounced results of antifungal activity in contrast to their corresponding Schiff base ligands.

Table 4. Antifungal Bioassay of Ligands **(L^1)-(L^3)** and Metal(II) Complexes **(1)-(12)**

Antifungal activity, % Inhibition								
Compounds	**(a)**	**(b)**	**(c)**	**(d)**	**(e)**	**(f)**	**(SA)**	**Average**
(L^1)	50	57	48	00	43	54	21.14	42.0
(L^2)	44	56	00	41	00	47	24.78	31.3
(L^3)	55	00	44	46	38	29	19.35	35.3
(1)	62	67	58	25	56	73	16.77	41.8
(2)	25	35	45	27	36	20	9.05	31.3
(3)	54	45	62	25	43	69	15.62	49.7
(4)	75	26	64	52	17	57	22.47	48.5
(5)	42	38	43	57	25	67	14.76	45.5
(6)	66	39	27	68	29	76	21.65	50.8
(7)	44	73	77	58	56	49	12.59	59.7
(8)	46	48	68	55	28	12	19.94	42.8
(9)	50	45	10	61	42	34	17.35	40.33
(10)	45	25	57	33	40	11	16.06	35.17
(11)	29	34	10	35	52	65	19.05	37.50
(12)	70	38	28	43	33	60	16.34	45.33

Average Activity of Ligands **(L^1)-(L^3)** = 36.2 %; Average Activity of Complexes **(1)-(12)** = 44.03 %; **(a)** *T. Mentogrophytes* **(b)** *E. Floccosum* **(c)** *A. Niger* **(d)** *M. Canis* **(e)** *F. Culmorum* **(f)** *T. Schoenleinii*; weaker = 0-33 %, moderate = 34-54 %, 55-100 % = significant, **SA** = Statistical Analysis.

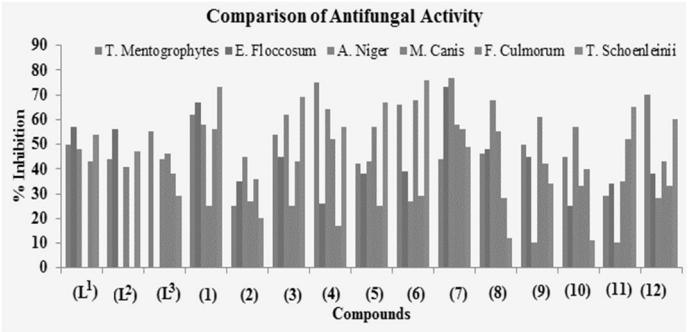


Fig. 2. Comparison of Antifungal Activity of Schiff bases versus Metal(II) Complexes

Minimum Inhibitory Concentration (MIC)

The selected synthetic ligands and their divalent metal chelates showed a good bactericidal activity more than 80 % for the minimum inhibitory concentration (MIC) studies and the obtained outcomes of this assay are shown in **Table 5**. The results of antibacterial activity assay have shown that the complexes of metals(II) as **(1)-(3), (6), (7), (11) and (12)** have exhibited more than 80 % activity. And due to this reason, these metal(II) complexes were selected as well as investigated for MIC studies. The MIC values of these tested metal(II) complexes ranged from 22.18-47.22 µg/mL. Among these, the compound **(12)** was highly active showing strong inhibition at 22.18 µg/mL concentration against *Klebsiella pneumoniae* bacterial strain.

Table 5. Minimum Inhibitory Concentration (μgmL^{-1}) of the Tested Metal(II) Complexes **(1)-(3), (6), (7)** and **(11)-(12)** against the Pathogenic Bacterial Strains.

No.	*E. Coli*	*S. Faecalis*	*P. Aeruginosa*	*K. Pneumoniae*	*S. Aureus*	*B. Subtilis*
(1)	35.12	41.61	–	–	–	–
(2)	29.17	37.19	39.24	–	33.23	–
(3)	–	47.22	–	–	–	–
(6)	–	–	26.61	–	–	41.32
(7)	–	36.35	–	–	–	25.27
(11)	–	24.35	40.53	–	–	–
(12)	38.16	–	–	22.18	–	–

CONCLUSION

In this study, new series of Schiff bases were synthesized through condensation reaction of ethylene-1,2-diamine with benzaldehyde, 2-chlorobenzaldeyhde and 4-chloroacetophenone in molar ratio of 1:2, respectively. The molecular structures of these newly synthesized ligands were spectroscopically illustrated by UV-Vis, FT-IR, ^{1}HNMR, ^{13}CNMR, elemental and mass spectral analysis. These ligands acted as neutral and bidentate moieties, and coordinated with the divalent metal ions through NNO donor sites to derive 3*d*-metal complexes. Some spectral as well as analytical methods were used to verify the proposed structures of these synthesized metal complexes having the overall formulae $[M(L)_2.2H_2O]Cl_2$. Based on the spectral as well as magnetic moment results, octahedral geometry was suggested for all the metal complexes. All the compounds were evaluated for antibacterial and antifungal activities against selected bacterial and fungal strains, respectively. The results of these biological inhibitions revealed that metal complexes have shown enhanced activities in comparison to the corresponding uncomplexed ligands. Zn(II) complex **(12)** of ligand **(L^3)** was proved as the most active antibacterial complex, while the Cu(II) complex **(7)** of ligand **(L^2)** was concluded as the most active antifungal complex. This improved activity was due to chelation effect that has reduced the polarity of metal ions by complexing with the ligands.

DISCLOSURE

No potential conflict of interest was reported by authors.

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