APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2007; **21**: 826–835
Published online 31 July 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI:10.1002/aoc.1299

Synthesis, characterization and reactivity towards first-row d-transition metals and biological significance of new pyridinyl derived N-substituted sulfonamides

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Received 25 April 2007; Revised 28 May 2007; Accepted 29 May 2007

Cobalt (II), copper (II), nickel (II) and zinc (II) complexes of new pyridinyl-derived N-substituted sulfonamides were synthesized. The nature of bonding and the structure of compounds were deduced from elemental analyses, molar conductances, magnetic moments, IR, ^{1}H NMR, ^{13}C NMR and electronic spectral data. An octahedral geometry has been suggested for the complexes. Complexes along with their ligands were assessed for their antibacterial and antifungal activities on six species of pathogenic bacteria (*Escherichia coli, Shigella flexeneri, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Bacillus subtilis*) and fungi (*Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata*). The results showed that all the compounds have moderate to significant antibacterial activity which was, in many cases, enhanced on chelation. Similar results were observed for antifungal activity. Brine shrimp bioassay was also carried out for *in vitro* cytotoxic properties against *Artemia salina*. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: sulfonamides; metal (II) complexes; antibacterial; antifungal; cytotoxicity

INTRODUCTION

Sulfonamides constitute an important class of drugs[1] with several types of pharmacological actions. Among sulfonamides, N-substituted sulfonamides are recognized as having antibacterial,[2,3] antitumor,[4] diuretic,[5] anti-carbonic anhydrase,[6,7] hypoglycaemic[8] and anti-thyroid[9] properties and as protease inhibitors.[10] The past decade has perceived an upsurge of interest in metal-based therapeutics for both diagnosis and treatment of diseases. The most significant part of such metal-based drug chemistry is the ability of metal ions to bind *in vivo* with proteins and peptides. Of great interest, simple and N-substituted sulfonamides have been observed to attract much attention in this emerging area of metal-based sulfa drugs. It was initially stimulated by the successful introduction of metal complexes of sulfadiazine to prevent bacterial infections.[11,12] These metal complexes employ themselves to slow release of metal ions[13] from the source, exclusively dependent on its binding nature. It is, therefore, vital to understand the coordination behaviour and relationship of metals in biological systems. In view of the versatile importance of sulfonamides and to identify their coordination properties, we began a program,[14–16] in synthesizing and designing various metal-based sulfonamides and exploring their structural and biological chemistry. In the same continuation, we herein describe the preparation and characterization of Co(II), Cu(II) Ni(II) and Zn(II) complexes with pyridinyl-derived N-substituted sulfonamides of the types sulfamethazine, sulfisoxazole, sulfamethaxazole and sulfathiazole. Also, *in vitro* antibacterial, antifungal and cytotoxic properties of these synthesized sulfonamides in comparison to their metal complexes have been evaluated and reported.

EXPERIMENTAL

All reagents and solvents used were of analytical grades; all metals (II) were used as chloride salts. IR spectra

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Contract/grant sponsor: Higher Education Commission, Government of Pakistan; Contract/grant number: 20-16/Acad(R&D)/2nd Phase/03/211.

were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer. NMR spectra were recorded on Perkin-Elmer 283B spectrometer. UV–visible spectra were obtained in DMF (dimethylformamide) solvent on a Hitachi U-2000 double-beam spectrophotometer. C, H and N analyses, conductance and magnetic measurements were carried out on solid compounds using the respective instruments. *In-vitro* antibacterial, antifungal and cytotoxic properties were studied at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Synthesis of Ligands

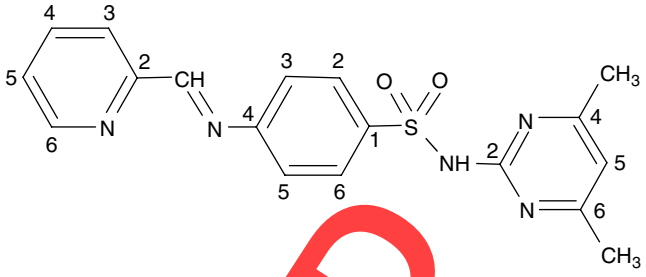
N-(4,6-dimethylpyrimidin-2-yl)-4-[(pyridin-2-ylmethylene)amino]-benzenesulfonamide
(L$_1$)

To an ethanolic (30 ml) solution of sulfamethazine (1.95 g, 0.007 mol), pyridine-2-carbaldehyde (0.75 g, 0.67 ml, 0.007 mol) solution in ethanol (15 ml) was added with stirring. The solution was refluxed for 3 h. The precipitates formed during reflux were cooled to room temperature and collected by suction filtration. Washing thoroughly with ethanol (2 × 10 ml) afforded TLC pure product (2.03 g, 79% yield). The same method was applied to prepare all other ligands (**L$_2$ – L$_4$**).

Physical measurements, analytical estimations and spectral properties of the ligands and zinc (II) complexes

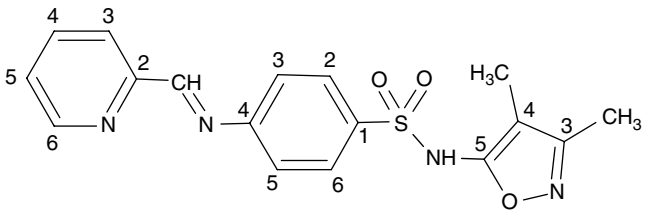
N-(4,6-dimethylpyrimidin-2-yl)-4-[(pyridin-2-ylmethylene)amino]-benzenesulfonamide
(L$_1$)

Yield 79% (2.03 g); m.p. 225–27 °C; IR (KBr, cm^{-1}): 3238 (NH), 1593 (HC=N), 1395 (C–N), 1540 (–N=pyrimidine ring), 1325, 1120 (S=O), 955 (S–N), 835 (C–S); ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.25 (s, 6H, CH$_3$), 6.74 (s, 1H, pyrimidine), 7.50–7.85 (m, 4H, N-Ph), 8.01–8.23 (m, 4H, pyridine), 9.30 (s, 1H, azomethine), 11.34 (s, 1H, SO$_2$NH); ^{13}C NMR (δ, ppm): 25.1 (2CH$_3$-pyrimidine), 165.2 (C$_4$,C$_6$-pyrimidine), 103.0 (C$_5$-pyrimidine), 168.5 (C$_2$-pyrimidine), 138.2 (C$_1$-phenyl), 128.6 (C$_2$,C$_6$-phenyl), 122.6 (C$_3$,C$_5$-phenyl), 156.4 (C$_4$-phenyl), 158.0 (C=N, azomethine), 148.6 (C$_2$-pyridine), 132.2–143.1 (C$_3$,C$_4$,C$_5$-pyridine); anal. calcd for C$_{18}$H$_{17}$N$_5$O$_2$S (367.42): C, 58.84; H, 4.66; N, 19.06; found: C, 58.78; H, 4.77; N, 19.03%. ^{1}H NMR of Zn (II) complex (DMSO-d$_6$, δ, ppm): 2.25 (s, 6H, CH$_3$), 6.74 (s, 1H, pyrimidine), 7.65–7.95 (m, 4H, N-Ph), 8.25–8.45 (m, 4H, pyridine), 9.55 (s, 1H, azomethine), 11.34 (s, 1H, SO$_2$NH). ^{13}C NMR of Zn (II) complex (δ, ppm): 25.1 (CH$_3$-pyrimidine), 165.2 (C$_4$, C$_6$-pyrimidine), 103.0 (C$_5$-pyrimidine), 168.5 (C$_2$-pyrimidine), 138.2 (C$_1$-phenyl), 128.6 (C$_2$,C$_6$-phenyl), 122.6 (C$_3$,C$_5$-phenyl), 165.2 (C$_4$-phenyl), 172.3 (C=N, azomethine), 154.6 (C$_2$-pyridine), 132.2–143.1 (C$_3$,C$_4$,C$_5$-pyridine).



N-(3,4-dimethylisoxazol-5-yl)-4-[(pyridin-2-ylmethylene)amino]-benzenesulfonamide
(L$_2$)

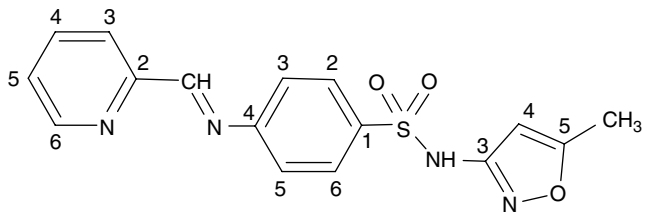
Yield 78% (1.95 g); m.p. 263–65 °C; IR (KBr, cm^{-1}): 3239 (NH), 1593 (HC=N), 1395 (C–N), 1547 (–N=isoxazole ring), 1325, 1120 (S=O), 955 (S–N), 835 (C–S); ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.35 (m, 6H, CH3), 7.50–7.85 (m, 4H, N-Ph), 8.01–8.23 (m, 4H, pyridine), 9.30 (s, 1H, azomethine), 11.34 (s, 1H, SO$_2$NH); ^{13}C NMR (δ, ppm): 15.1 (CH$_3$-isoxazole), 9.5 (CH$_3$-isoxazole), 159.9 (C$_3$-isoxazole), 100.5 (C$_4$-isoxazole), 158.9 (C$_5$-isoxazole), 138.2 (C$_1$-phenyl), 128.6 (C$_2$,C$_6$-phenyl), 122.6 (C$_3$,C$_5$-phenyl), 156.4 (C$_4$-phenyl), 158.0 (C=N, azomethine), 148.6 (C$_2$-pyridine), 132.2–143.1 (C$_3$,C$_4$,C$_5$-pyridine); anal. calcd for C$_{17}$H$_{16}$N$_4$O$_3$S (356.40): C, 57.29; H, 4.52; N, 15.72; found: C, 57.34; H, 4.55; N, 15.66%. ^{1}H NMR of Zn (II) complex (DMSO-d$_6$, δ, ppm): 2.35 (m, 6H, CH$_3$), 7.65–7.95 (m, 4H, N-Ph), 8.25–8.45 (m, 4H, pyridine), 9.55 (s, 1H, azomethine), 11.34 (s, 1H, SO$_2$NH). ^{13}C NMR of Zn (II) complex (δ, ppm): 15.1 (CH$_3$-isoxazole), 9.5 (CH$_3$-isoxazole), 159.9 (C$_3$-isoxazole), 100.5 (C$_4$-isoxazole), 158.9 (C$_5$-isoxazole), 138.2 (C$_1$-phenyl), 128.6 (C$_2$,C$_6$-phenyl), 122.6 (C$_3$,C$_5$-phenyl), 165.2 (C$_4$-phenyl), 172.3 (C=N, azomethine), 154.6 (C$_2$-pyridine), 132.2–143.1(C$_3$,C$_4$,C$_5$-pyridine).



N-(5-methylisoxazol-3-yl)-4-[(pyridin-2-ylmethylene)amino]-benzenesulfonamide
(L$_3$)

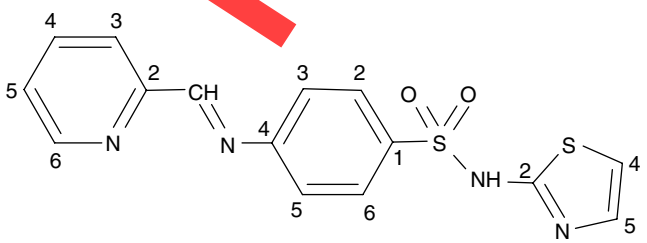
Yield 71% (1.70 g); m.p. 281–83 °C; IR (KBr, cm^{-1}): 3238 (NH), 1593 (HC=N), 1395 (C–N), 1547 (–N=isoxazole ring), 1325, 1120 (S=O), 955 (S–N), 835 (C–S); ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.29 (s, 3H, CH3), 6.09 (s, 1H, isoxazole), 7.50–7.85 (m, 4H, N-Ph), 8.01–8.23 (m, 4H, pyridine), 9.30 (s, 1H, azomethine), 11.34 (s, 1H, SO$_2$NH); ^{13}C NMR (δ, ppm): 12.8 (CH$_3$-isoxazole), 159.6 (C$_5$-isoxazole), 95.0 (C$_4$-isoxazole), 150.0 (C$_3$-isoxazole), 138.2 (C$_1$-phenyl), 128.6 (C$_2$,C$_6$-phenyl), 122.6 (C$_3$,C$_5$-phenyl), 156.4 (C$_4$-phenyl),

158.0 (C═N, azomethine), 148.6 (C_2-pyridine), 132.2–143.1 (C_3,C_4,C_5-pyridine); anal. calcd for $C_{16}H_{14}N_4O_3S$ (342.37): C, 56.13; H, 4.12; N, 16.36; found: C, 56.17; H, 4.18; N, 16.28%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 2.29 (s, 3H, CH3), 6.09 (s, 1H, isoxazole), 7.65–7.95 (m, 4H, N-Ph), 8.25–8.45 (m, 4H, pyridine), 9.55 (s, 1H, azomethine), 11.34 (s, 1H, SO_2NH). ^{13}C NMR of Zn (II) complex (δ, ppm): 12.8 (CH_3-isoxazole), 159.6 (C_5-isoxazole), 95.0 (C_4-isoxazole), 150.0 (C_3-isoxazole), 138.2 (C_1-phenyl), 128.6 (C_2,C_6-phenyl), 122.6 (C_3,C_5-phenyl), 165.2 (C_4-phenyl), 172.3 (C═N, azomethine), 154.6 (C_2-pyridine), 132.2–143.1(C_3,C_4,C_5-pyridine).



*N-(1,3-thiazol-2-yl)-4-[(pyridin-2-ylmethylene)amino]benzenesulfonamide (**L**$_4$)*

Yield 75% (1.81 g); m.p. 237–39 °C; IR (KBr, cm^{-1}): 3237 (NH), 1593 (HC═N), 1395 (C–N), 1543 (–N═thiazol ring), 1325, 1120 (S═O), 955 (S–N), 835 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.81–7.21 (m, 2H, thiazol), 7.50–7.85 (m, 4H, N-Ph), 8.01–8.23 (m, 4H, pyridine), 9.30 (s, 1H, azomethine), 11.34 (s, 1H, SO_2NH); ^{13}C NMR (δ, ppm): 108.0 (C_4-thiazol), 138.3 (C_5-thiazol), 171.7 (C_2-thiazol), 138.2 (C_1-phenyl), 128.6 (C_2,C_6-phenyl), 122.6 (C_3,C_5-phenyl), 156.4 (C_4-phenyl), 158.0 (C═N, azomethine), 148.6 (C_2-pyridine), 132.2–143.1 (C_3,C_4,C_5-pyridine); anal. calcd for $C_{15}H_{12}N_4O_2S_2$ (344.41): C, 52.31; H, 3.51; N, 16.27; found: C, 52.28; H, 3.62; N, 16.23%. 1H NMR of Zn (II) complex (DMSO-d6, δ, ppm): 6.81–7.21 (m, 2H, thiazol), 7.65–7.95 (m, 4H, N-Ph), 8.25–8.45 (m, 4H, pyridine), 9.55 (s, 1H, azomethine), 11.34 (s, 1H, SO_2NH). ^{13}C NMR of Zn (II) complex (δ, ppm): 108.0 (C_4-thiazol), 138.3 (C_5-thiazol), 171.7 (C_2-thiazol), 138.2 (C_1-phenyl), 128.6 (C_2,C_6-phenyl), 122.6 (C_3,C_5-phenyl), 165.2 (C_4-phenyl), 172.3 (C═N, azomethine), 154.6 (C_2-pyridine), 132.2–143.1(C_3,C_4,C_5-pyridine).



Synthesis of metal (II) complexes

*Synthesis of Co (II) complex with N-(4,6-dimethylpyrimidin-2-yl)-4-[(pyridin-2-ylmethylene)amino]-benzenesulfonamide [Co(L$_1$)$_2$Cl$_2$] (**1**)*

To a hot magnetically stirred dioxane (10 ml) solution of *N*-(4,6-dimethylpyrimidin-2-yl)-4-[(pyridin-2-ylmethylene) amino]-benzenesulfonamide (**L**$_1$) (0.74 g, 0.002 mol), an aqueous solution (15 ml) of Co (II) $Cl_2.6H_2O$ (0.24 g, 0.001 mol) was added. The mixture was refluxed for 1 h, 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 which was filtered, washed with dioxane (2 × 5 ml) then with ether and dried. Recrystallization from 50% aqueous dioxane gave the desired product. Unfortunately only microcrystalline powders could be obtained, which could not to be used for X-ray structural determinations.

The same method was used for the preparation of all other complexes (**2–16**).

Biological properties

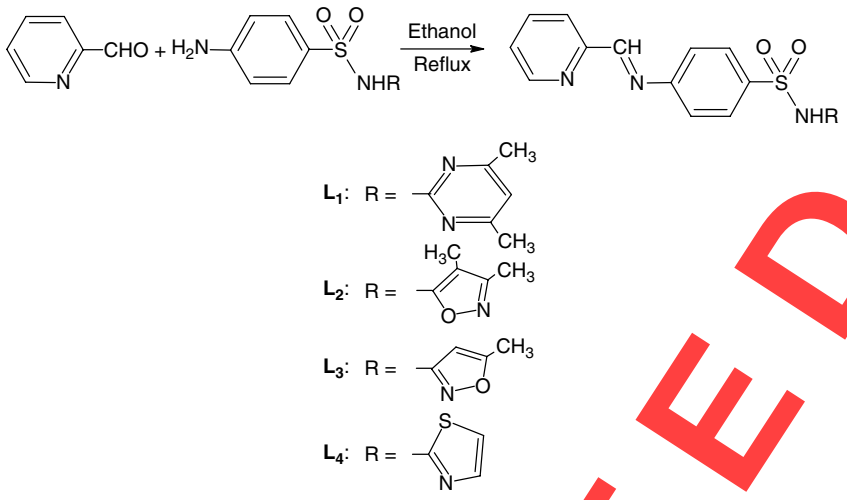
Antibacterial bioassay (in vitro)

All the synthesized compounds (**L**$_1$ – **L**$_4$) and metal (II) complexes (**1–16**) were screened *in vitro* for their antibacterial activity against four Gram-negative (*E. coli, S. flexenari, P. aeruginosa* and *S. typhi*) and two Gram-positive (*S. aureus* and *B. subtilis*) bacterial strains by the agar-well diffusion method.[17,18] The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centres at least 24 mm apart. Two- to eight-hour-old bacterial inocula containing approximately 10^4–10^6 colony-forming units (CFU/ml) were spread on the surface of the nutrient agar with the help of a sterile cotton swab. The recommended concentration of the test sample (1 mg/ml in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenum 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 participating role 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.

Antifungal activity (in vitro)

All compounds were studied against six fungal cultures for antifungal activities. Sabouraud dextrose agar (Oxoid, Hampshire, UK) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspensions and transferred to Petri plates. Discs soaked in 20 ml (200 µg/ml in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32 °C for 7 days. The results were recorded[19] as percentage of inhibition and compared with standard drugs miconazole and amphotericin B.

DOI: 10.1002/aoc



Scheme 1. Preparation of ligands.

Minimum inhibitory concentration

Compounds containing high antibacterial activity (over 80%) were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion technique by preparing discs containing 10, 25, 50 and 100 μg/ml of the compounds and applying the protocol.[20]

Cytotoxicity (in vitro)

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with 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 matter compartment was opened to ordinary light. After 2 days nauplii were collected using 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 DMF. From this stock solutions 500, 50 and 5 μg/ml 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 DMF only. The solvent was allowed to evaporate overnight. After 2 days, when shrimp larvae were ready, 1 ml of sea water and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 ml per vial. After 24 h the number of survivors was counted. Data were analysed using a Finney computer program to determine the LD_{50} values.[21,22]

RESULTS AND DISCUSSION

Chemistry, composition and characterization of the ligands

The sulfonamide derived ligands ($\mathbf{L_1} - \mathbf{L_4}$) were prepared as shown in Scheme 1. All ligands were only soluble in dioxane, DMF and DMSO. The composition of the ligands is consistent with their microanalytical data.

Chemistry, composition and characterization of the metal (II) complexes

The metal (II) complexes (**1–16**) of the ligands ($\mathbf{L_1} - \mathbf{L_4}$) were prepared (Fig. 1) according to the following equation:

$$MCl_2 + 2\text{Ligand(L)} \longrightarrow [M(L)_2(Cl)_2]$$

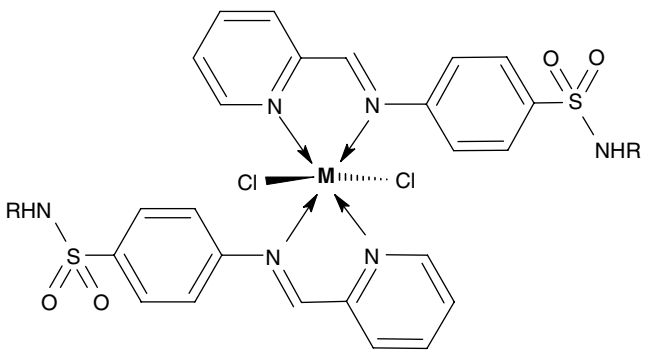
$$M = \text{Co(II), Cu(II), Ni(II)and Zn(II)}$$

$$L = (\mathbf{L_1} - \mathbf{L_4})$$

Physical measurements and analytical data for complexes (**1–16**) are given in Table 1.

Conductance and magnetic susceptibility measurements

The molar conductance values (in DMF) for complexes (**1–16**) fall within the range 13.5–21.2 Ω^{-1} cm^2 mol^{-1} for all



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

Figure 1. Proposed structure of the metal (II) complex.

DOI: 10.1002/aoc

Table 1. Physical measurements and analytical data of the metal (II) complexes

No.		m.p. (dec.) (°C)	Yield (%)	Calcd (found)%		
				C	H	N
1.	$[Co(L_1)_2(Cl)_2]$ [864.70] $C_{36}H_{34}N_{10}O_4S_2Cl_2Co$	275–278	80	50.01 (50.09)	3.96 (3.93)	16.20 (16.12)
2.	$[Cu(L_1)_2(Cl)_2]$ [869.31] $C_{36}H_{34}N_{10}O_4S_2Cl_2Cu$	273–276	81	49.74 (49.77)	3.94 (3.87)	16.11 (16.14)
3.	$[Ni(L_1)_2(Cl)_2]$ [864.45] $C_{36}H_{34}N_{10}O_4S_2Cl_2Ni$	276–279	84	50.02 (50.06)	3.96 (3.92)	16.20 (16.22)
4.	$[Zn(L_1)_2(Cl)_2]$ [871.15] $C_{36}H_{34}N_{10}O_4S_2Cl_2Zn$	273–276	80	49.63 (49.69)	3.93 (3.90)	16.08 (16.13)
5.	$[Co(L_3)_2(Cl)_2]$ [842.64] $C_{34}H_{32}N_8O_6S_2Cl_2Co$	266–269	83	48.46 (48.48)	3.83 (3.89)	13.30 (13.34)
6.	$[Cu(L_3)_2(Cl)_2]$ [847.26] $C_{34}H_{32}N_8O_6S_2Cl_2Cu$	273–276	81	48.20 (48.17)	3.81 (3.87)	13.23 (13.18)
7.	$[Ni(L_3)_2(Cl)_2]$ [842.40] $C_{34}H_{32}N_8O_6S_2Cl_2Ni$	263–266	85	48.48 (48.47)	3.83 (3.82)	13.30 (13.35)
8.	$[Zn(L_3)_2(Cl)_2]$ [849.10] $C_{34}H_{34}N_6O_8S_2Cl_2Zn$	273–276	80	48.09 (48.06)	3.80 (3.82)	13.20 (13.22)
9.	$[Co(L_4)_2(Cl)_2]$ [814.59] $C_{32}H_{28}N_8O_6S_2Cl_2Co$	267–270	83	47.18 (47.16)	3.46 (3.48)	13.76 (13.69)
10.	$[Cu(L_4)_2(Cl)_2]$ [819.20] $C_{32}H_{28}N_8O_6S_2Cl_2Cu$	277–280	81	46.92 (46.82)	3.45 (3.47)	13.68 (16.58)
11.	$[Ni(L_4)_2(Cl)_2]$ [814.35] $C_{32}H_{28}N_8O_6S_2Cl_2Ni$	268–271	85	47.20 (47.27)	3.47 (3.42)	13.76 (13.75)
12.	$[Zn(L_4)_2(Cl)_2]$ [821.05] $C_{32}H_{28}N_8O_6S_2Cl_2Zn$	263–266	80	46.81 (46.86)	3.44 (3.42)	13.65 (13.59)
13.	$[Co(L_5)_2(Cl)_2]$ [818.67] $C_{30}H_{24}N_8O_4S_4Cl_2Co$	282–285	83	44.01 (44.06)	2.95 (2.98)	13.69 (13.64)
14.	$[Cu(L_5)_2(Cl)_2]$ [823.28] $C_{30}H_{24}N_8O_4S_4Cl_2Cu$	287–290	81	43.77 (43.72)	2.94 (2.97)	13.61 (13.68)
15.	$[Ni(L_5)_2(Cl)_2]$ [818.43] $C_{30}H_{24}N_8O_4S_4Cl_2Ni$	288–291	85	44.03 (44.07)	2.96 (2.92)	13.69 (13.65)
16.	$[Zn(L_5)_2(Cl)_2]$ [825.13] $C_{30}H_{24}N_8O_4S_4Cl_2Zn$	281–283	80	43.67 (43.66)	2.93 (2.92)	13.58 (13.52)

complexes, showing their non-electrolytic[23] nature. This in turn suggests that the chloride ions are coordinated with the metal ions. The room temperature magnetic moment values of the complexes are given in Table 2. The observed magnetic moment (4.93–4.99 BM) is consistent with half-spin octahedral cobalt (II) complexes. The magnetic moment values (1.87–1.91 BM) measured for the copper (II) complexes lie in the range expected for a d^9-system containing one unpaired electron with octahedral geometry.[24] The measured values (3.25–3.32 BM) for the nickel (II) complexes also suggest[25] their octahedral geometry. The zinc (II) complexes were found to be diamagnetic as expected.

IR spectra

The important IR spectral bands of the ligands and its metal complexes are given in the Experimental and in Table 2. All ligands contain various potential donor sites. In the IR spectra of the ligands a sharp band observed at 1593 cm^{-1} and medium sharp band at 1395 cm^{-1} were assigned[26] to the ν (C=N) mode and ν (C–N) stretching of pyridinyl ring, respectively. Evidence of the nitrogen bonding of the azomethine (C=N) group to the central metal atom stems from the shift of the ν (C=N) frequency lower by 23–30 cm^{-1} (1563–1570 cm^{-1}) in all of the complexes. This is further confirmed by the appearance of the new bands at 434–439 cm^{-1} due to the ν (M–N) band.[27]

The coordination through the pyridinyl ring nitrogen is revealed by shifting of the C–N band to much lower frequencies (1347–1353 cm^{-1}) in all the complexes as compared with that of the ligands. This is further confirmed by the appearance of the new band at 528–538 cm^{-1} due to ν (M–N) in all the complexes. The bands in the ligand due to $\nu_{asymm}(SO_2)$ and $\nu_{symm}(SO_2)$ appear at 1325 and 1120 cm^{-1}, respectively.[28] These bands remain unchanged in

DOI: 10.1002/aoc

Table 2. Analytical conductivity, magnetic and spectral data of metal (II) complexes

No.	Ω_M (Ω^{-1} cm^2 mol^{-1})	BM (μ_{eff})	λ_{max} (cm^{-1})	IR (cm^{-1})
1.	13.9	4.94	7355,17445, 20585,29315	1568(C=N),1325,1120(SO_2),532(M–N),325(M–Cl), 955(S–N),835(C–S),435(M–N),1348(C–N)
2.	16.7	1.89	14995,19140, 30375	1570(C=N),1325,1120(SO_2),536(M–N),325(M–Cl), 955(S–N),835(C–S),439(M–N),1347(C–N)
3.	15.9	3.32	10350,15765, 26675,29870	1564(C=N),1325,1120(SO_2),528(M–N),325(M–Cl), 955(S–N),835(C–S),436(M–N),1353(C–N)
4.	18.7	Dia	28935	1567(C=N),1325,1120(SO_2),536(M–N),325(M–Cl), 955(S–N),835(C–S),438(M–N),1349(C–N)
5.	13.5	4.99	7275,17355, 20505,29370	1564(C=N),1325,1120(SO_2),534(M–N),325(M–Cl), 955(S–N),835(C–S),434(M–N),1348(C–N)
6.	13.7	1.89	14985,19180, 30355	1566(C=N),1325,1120(SO_2),538(M–N),325(M–Cl), 955(S–N),835(C–S),434(M–N),1347(C–N)
7.	14.5	3.28	10405,15690, 26325,29995	1567(C=N),1325,1120(SO_2),530(M–N),325(M–Cl), 955(S–N),835(C–S),437(M–N),1350(C–N)
8.	15.9	Dia	28530	1570(C=N),1325,1120(SO_2),528(M–N),325(M–Cl), 955(S–N),835(C–S),438(M–N),1351(C–N)
9.	16.0	4.96	7305,17495, 20680,29395	1570(C=N),1325,1120(SO_2),537(M–N),325(M–Cl), 955(S–N),835(C–S),439(M–N),1350(C–N)
10.	17.8	1.91	14985,19180, 30335	1566(C=N),1325,1120(SO_2),535(M–N),325(M–Cl), 955(S–N),835(C–S),438(M–N),1352(C–N)
11.	14.2	3.26	10425,15865, 26535,29995	1567(C=N),1325,1120(SO_2),536(M–N),325(M–Cl), 955(S–N),835(C–S),435(M–N),1351(C–N)
12.	16.3	Dia	28980	1567(C=N),1325,1120(SO_2),538(M–N),325(M–Cl), 955(S–N),835(C–S),437(M–N),1347(C–N)
13.	13.5	4.97	7405,17495, 20445,29285	1565(C=N),1325,1120(SO_2),531(M–N),325(M–Cl), 955(S–N),835(C–S),434(M–N),1350(C–N)
14.	13.7	1.87	14720,19190, 30380	1563(C=N),1325,1120(SO_2),530(M–N),325(M–Cl), 955 (S–N),835(C–S),436(M–N),1348(C–N)
15.	14.5	3.29	10455,15610, 26595,29850	1569(C=N),1325,1120(SO_24),537(M–N),325(M–Cl), 955(S–N),835(C–S),439(M–N),1351(C–N)
16.	15.9	Dia	28980	1570(C=N),1325,1120(SO_2),528(M–N),325(M–Cl), 955(S–N),835(C–S),434(M–N),1347(C–N)

the complexes, indicating that this group is not participating in coordination. This is supported by the unchanged ν (S–N) and ν (C–S) modes appearing between 955 and 835 cm^{-1}, respectively,[29] in the ligands after complexation. Also, the band due to ν (–N=) pyrimidine, isoxazole or thiazol ring do not show any appreciable change on complexation, suggesting that the ring nitrogens of these moieties are not taking part in coordination. A new band appearing at 325 cm^{-1} assigned[30] to the ν (M–Cl) mode in all the metal complexes was however, indicative of the fact that chloride atoms are coordinated with the central metal atom.

^{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 are recorded in the Experimental. All the protons due to heteroaromatic/aromatic groups were found to be in their expected region.[31] The conclusions drawn from these studies lend further support to the mode of bonding discussed in their IR spectra. The coordination of the azomethine nitrogen is inferred by the downfield shifting of the –CH=N–proton signal from 9.30 ppm in the ligand to 9.55 ppm in the complexes. Protons surrounding the coordination sites underwent downfield shifting by 0.10–0.25 ppm due to the increased conjugation[32] and coordination with the metal atoms. Furthermore, the number of protons calculated from the integration curves and those obtained from the values of the expected CHN analyses agree well with each other.

^{13}C NMR spectra

^{13}C NMR spectra of the free ligands and their diamagnetic zinc (II) complexes were also recorded in DMSO-d_6. The ^{13}C NMR spectral data along with the possible assignments

DOI: 10.1002/aoc

were recorded in the Experimental. The carbons atoms due to heteroaromatic/aromatic groups were found as to be in their expected region. The conclusions drawn from these studies present further support to the mode of bonding discussed in their IR and ^{1}H NMR spectra. Downfield shifting of the –CH=N– signal from 158.0 ppm in the ligand to 172.3 ppm in its metal (II) complexes revealed coordination of the azomethine nitrogen to the metal atom. Carbons surrounding the coordination sites underwent downfield shifting by 6.0–14.3 ppm due to the increased conjugation and coordination with the metal atoms. Furthermore, the presence of the number of carbons agree well with the expected values.

Electronic spectra

The Co(II) complexes exhibited well-resolved, low-energy bands at 7275–7485 cm^{-1}, 17 355–17 520 cm^{-1} and a strong high-energy band at 20 445–20 680 cm^{-1} (Table 2) which are assigned[24] to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ in an octahedral geometry.[25] A high intensity band at 29 285–29 395 cm^{-1} was assigned to the metal to ligand charge transfer. The magnetic susceptibility measurements for the solid Co (II) complexes are also indicative of three unpaired electrons per Co (II) ion, suggesting[33] consistency with their octahedral environment.

The electronic spectra of the Cu (II) complexes (Table 2) showed two low-energy weak bands at 14 720–15 160 and 19 140–19 315 cm^{-1} and a strong high-energy band at 30 335–30 380 cm^{-1} and may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively.[34] The strong high-energy band, in turn, is assigned to metal → ligand charge transfer. Also, the magnetic moment values for the copper (II) are indicative of anti-ferromagnetic spin–spin interaction through molecular association indicative of their octahedral geometry.[25]

The electronic spectra of the Ni (II) complexes (Table 2) showed d–d bands in the regions 10 350–10 490, 15 610–15 865 and 26 325–26 675 cm^{-1}. These are assigned[33] to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$, respectively, consistent with their well-defined octahedral configuration. The band at 29 850–30 225 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements showed two unpaired electrons per Ni (II) ion, suggesting[34] also an octahedral geometry for the Ni (II) complexes. The electronic spectra of the Zn (II) complexes (Table 2) exhibited only a high-intensity band at 28 530–29 145 cm^{-1} and were assigned[35] to a ligand–metal charge transfer.

Biological activity

Antibacterial bioassay (in vitro)

All compounds were tested against four Gram-negative (*E. coli*, *S. flexenari*, *P. aeruginosa* and *S. typhi*) and two Gram-positive (*S. aureus* and *B. subtilis*) bacterial strains (Table 3) according to the literature protocol.[18,19] The results were compared with those of the standard drug imipenum (Fig. 2). All ligands showed moderate to significant activity against all Gram-negative and Gram-positive bacterial strains except the activity of all compounds against strain *b* where no moderate to significant activity was observed. Compounds **1–16** exhibited overall a significant activity against *E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus* and *B. subtilis*. However a moderate activity was observed by compounds $\mathbf{L}_1$,$\mathbf{L}_2$,$\mathbf{L}_3$ and $\mathbf{L}_4$ against *c* and *d*, $\mathbf{L}_3$ and $\mathbf{L}_4$ against *e*, and $\mathbf{L}_1$,$\mathbf{L}_3$ and $\mathbf{L}_4$ against *f*. Weak to moderate activity was observed against *b*. Antibacterial activity was overall enhanced after complexation of the ligands. However the zinc (II) complexes of all the ligands were observed to be the most active against all species (Fig. 3).

Antifungal bioassay (in vitro)

The antifungal screening of all compounds was carried out against *T. longifusus*, *C. albican*, *A. flavus*, *M. canis*, *F. solani* and *C. glaberate* fungal strains according to the literature protocol.[19] All synthesized compounds showed good antifungal activity against different fungal strains. Compounds **15** and **16** showed good antifungal activity against all the fungal strains. The results of inhibition were compared with the results of inhibition of standard drugs

Table 3. Antibacterial bioassay (concentration used 1 mg/ml of DMSO) of ligands and metal (II) complexes

	Compound [zone of inhibition (mm)]																				
Bacteria	$\mathbf{L}_1$	$\mathbf{L}_2$	$\mathbf{L}_3$	$\mathbf{L}_4$	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	SD
Gram-negative																					
E. coli	17	16	16	17	19	12	20	24	20	19	22	24	18	17	18	22	20	19	22	24	30
S. flexenari	08	06	07	09	08	06	10	10	12	10	11	12	11	12	13	13	11	11	10	14	27
P. aeruginosa	14	15	13	15	18	19	18	21	18	19	20	24	17	18	16	21	18	19	20	23	26
S. typhi	12	13	14	15	20	17	18	22	20	19	20	21	18	18	18	20	20	17	18	24	27
Gram-positive																					
S. aureus	16	17	12	14	17	16	17	23	19	20	20	22	17	18	17	24	19	20	18	26	30
B. subtilis	15	19	15	14	20	18	15	18	19	20	19	23	15	16	15	20	19	20	19	25	28

10 <= weak; >10 = moderate; >16 = significant. SD = standard drug (imipenum).

DOI: 10.1002/aoc

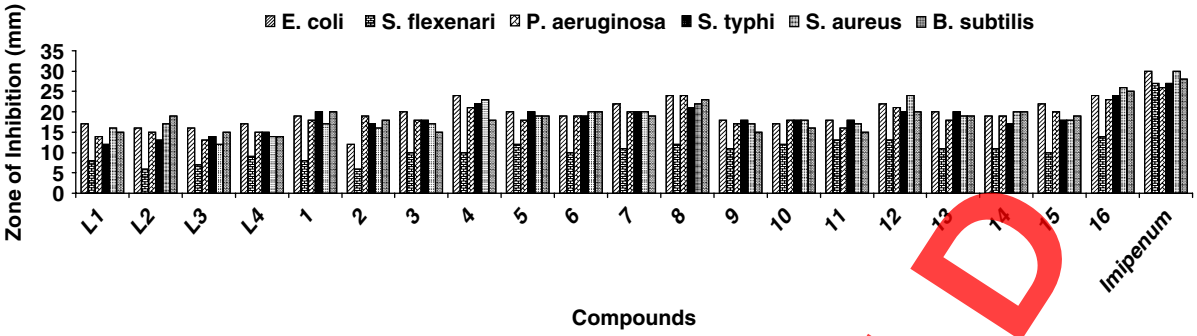


Figure 2. Comparison of antibacterial activity.

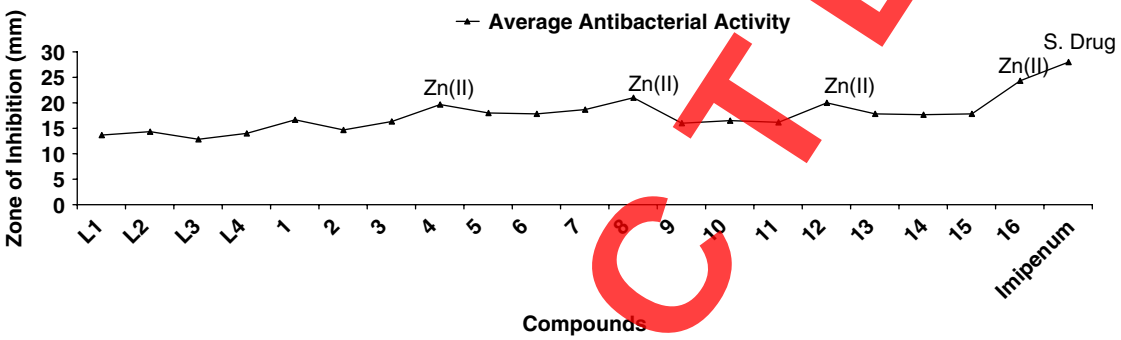


Figure 3. Average antibacterial activity of ligands vs metal (II) complexes.

Table 4. Antifungal bioassay (concentration used 200 μg/ml) of ligands and metal (II) complexes

	Compound																				
Organism	L_1	L_2	L_3	L_4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	SD
T. longifucus	70	40	80	00	50	60	40	00	55	35	40	20	45	65	00	00	78	30	45	25	A
C. albicans	55	00	00	65	50	00	60	00	75	45	35	60	40	00	00	75	65	60	65	60	B
A. flavus	80	70	80	00	60	80	00	40	65	00	55	55	35	40	40	40	45	45	60	50	C
M. canis	60	75	80	00	55	55	00	70	00	60	00	00	90	35	85	85	00	00	35	55	D
F. solani	00	90	20	20	40	45	70	90	00	90	00	70	00	50	30	00	00	20	80	85	E
C. glaberata	50	00	00	40	00	60	50	00	80	35	00	80	60	00	00	00	70	00	40	40	F

SD = standard drug MIC μg/ml: A = miconazole (70 μg/ml: 1.6822×10^{-7} M/ml), B = miconazole (110.8 μg/ml: 2.6626×10^{-7} M/ml), C = amphotericin B (20 μg/ml: 2.1642×10^{-8} M/ml), D = miconazole (98.4 μg/ml: 2.3647×10^{-7} M/ml), E = miconazole (73.25 μg/ml: 1.7603×10^{-7} M/ml), F = miconazole (110.8 μg/ml: 2.66266×10^{-7} M/ml).

miconazole and amphotericin B (Table 4) and individual synthesized compounds were also compared (Fig. 4). The effect of metal complexation on antifungal activity of the ligands can be seen (Fig. 5).

MIC for antibacterial activity

The preliminary antibacterial screening showed that compounds (**4,8,12** and **16**) were the most active ones (above 80%). These compounds were therefore selected for antibacterial MIC studies (Table 5).

Cytotoxic bioassay (in vitro)

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al.*[21] From the data recorded in Table 6, it is evident that five compounds (**2,6,10** and **14**) displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive for this assay. The compound **2** showed activity $LD_{50} = 5.982 \times 10^{-4}$ M/ml, compound **6** showed activity $LD_{50} = 8.533 \times 10^{-4}$ M/ml, compound **10** showed activity $LD_{50} = 6.116 \times 10^{-4}$ M/ml and compound **14** showed activity $LD_{50} = 5.770 \times 10^{-4}$ M/ml in

DOI: 10.1002/aoc

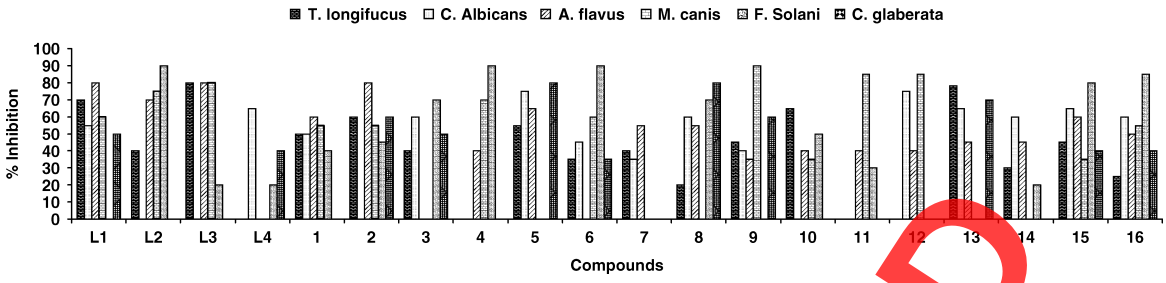


Figure 4. Comparison of antifungal activity.

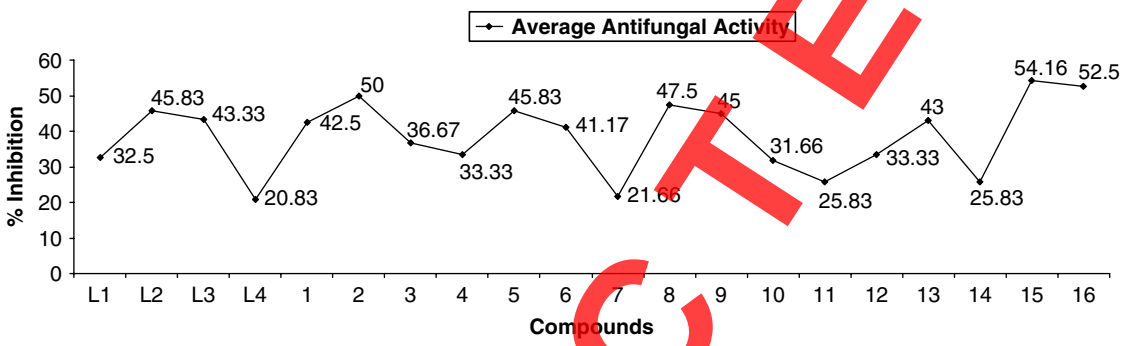


Figure 5. Average antifungal activity in ligands vs metal (II) complexes.

Table 5. Minimum inhibitory concentration (M/ml) of the selected compounds (**4, 8, 12** and **16**) against selected bacteria

No.	4	8	12	16
Gram-negative				
E. coli	1.148×10^{-7}	5.888×10^{-8}	—	3.030×10^{-8}
P. aeruginosa	5.740×10^{-8}	5.888×10^{-8}	1.218×10^{-7}	1.212×10^{-7}
S. typhi	1.148×10^{-7}	—	—	1.212×10^{-8}
Gram-positive				
S. aureus	—	—	3.045×10^{-8}	3.030×10^{-8}
B. subtilis	—	1.178×10^{-7}	—	6.060×10^{-8}

the present series of compounds. It was interesting to note that only copper complexes showed potent cytotoxicity whereas the other metal complexes did not. This activity relationship may help to serve as a basis for future direction towards the development of certain cytotoxic agents for clinical applications.

The enhancement of antibacterial/antifungal activity in ligands $\mathbf{L}_1 - \mathbf{L}_4$ upon chelation is rationalized on the basis of their structures and the mode of coordination/chelation. It has been suggested that chelation reduces the polarity of the metal ion[36–38] on partial sharing of its positive charge with the donor groups. The process of chelation increases the lipophilic nature of the metal atom, which in turn favours[39,40] its permeation through the lipoid layer of cell membrane of the micro-organism. It has also been suggested that some functional groups such as azomethine or heteroaromatics present in these compounds display[41,42] extensive biological activities that may be responsible for the increase of hydrophobic character and liposolubility of the molecules. It ultimately enhances activity of the compounds and biological utilization ratio.

CONCLUSION

The results of this investigation support the suggested structures of the pyridinyl derived sulfonamides and their metal (II) complexes. All the ligands are of bidentate nature. The geometry of all Co(II), Cu(II), Ni(II) and Zn(II) complexes is suggested to be octahedral, in which the two ligand molecules and two chlorine atoms participate (Fig. 1). All the synthesized pyridinyl derived sulfonamides and their

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Table 6. Brine shrimp bioassay data of the ligands $L_1 - L_4$ and their metal (II) complexes **1–16**

Compound	LD_{50} (M/ml)
L_1	$>2.722 \times 10^{-3}$
L_2	$>2.806 \times 10^{-3}$
L_3	$>2.921 \times 10^{-3}$
L_4	$>2.904 \times 10^{-3}$
1	$>1.156 \times 10^{-3}$
2	5.982×10^{-4}
3	$>1.157 \times 10^{-3}$
4	$>1.148 \times 10^{-3}$
5	$>1.187 \times 10^{-3}$
6	8.533×10^{-4}
7	$>1.187 \times 10^{-3}$
8	$>1.178 \times 10^{-3}$
9	$>1.228 \times 10^{-3}$
10	6.116×10^{-4}
11	$>1.228 \times 10^{-3}$
12	$>1.218 \times 10^{-3}$
13	$>1.221 \times 10^{-3}$
14	5.770×10^{-4}
15	$>1.222 \times 10^{-3}$
16	$>1.212 \times 10^{-3}$

metal (II) complexes have good antibacterial and antifungal properties.

Acknowledgement

We are thankful to the Higher Education Commission (HEC), Government of Pakistan for the financial assistance to carry out research project no. 20-16/Acad(R&D)/2nd Phase/03/211. We are also grateful to the HEJ Research Institute of Chemistry, University of Karachi for the help in undertaking NMR and biological assay.

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