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Structure and biological properties of first row d-transition metal complexes with N-substituted sulfonamides

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Abstract
Cobalt (II), copper (II), nickel (II) and zinc (II) complexes with 2-hydroxy-1-naphthaldehyde derived N-substituted sulfonamides have been synthesized and the nature of bonding and structure of compounds have been deduced from physical, analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR, Mass and electronic) data. An octahedral geometry has been suggested for the complexes. Complexes along with the ligands were assessed for their antibacterial and antifungal activities on different species of pathogenic bacteria and fungi. The results revealed the ligands to possess 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*.

Keywords: *Sulfonamides, metal (II) complexes, antibacterial, antifungal, cytotoxicity*

Introduction

N-Substituted sulfonamides are recognized as antibacterial [1–3], antitumor [4], diuretic [5], anti-carbonic anhydrase [6,7], hypoglycaemic [8], anti-thyroid [9] and protease inhibitors [10]. The past decade has perceived an upsurge interest in the 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 meticulous interest, simple and N-substituted sulfonamides have been observed to attract much attention into 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 slowly release of the metal ions [13] from the source exclusively dependent on the binding nature. In view of the versatile importance of sulfonamides and to identify their coordination properties we, have instigated a program [14–24], in synthesizing and designing various metal based sulphonamide 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 2-hydroxy-1-naphthaldehyde derived N-sulfonamides of the type sulfamethazine, sulfadiazine, sulfisoxazole, sulfamethaxazole, sulfathiazole and sulfaguanidin. Also, *in vitro* antibacterial, antifungal and cytotoxic properties of these synthesized sulfonamides in comparison to their metal complexes have been evaluated and reported in the present paper.

Experimental

Chemistry

All reagents and solvents used were of analytical grades; 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 d_6 DMSO as solvent. Infrared spectra of compounds were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer. The melting points of compounds were determined with a

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Gallenkamp melting point apparatus. *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-[(2-hydroxy-naphthalen-1-yl)methylene-amino]-benzenesulfonamide (L_1). To an ethanolic (30 mL) solution of sulfamethazine (1.95 g, 0.007 moles) 2-hydroxy-1-naphthaldehyde (1.21 g, 0.007 moles) in ethanol (15 mL) was added with stirring. The solution was refluxed for 2 h. The precipitates thus formed during refluxing, were cooled to room temperature and collected by suction filtration. Washing thoroughly with ethanol (2 × 10 ml), afforded TLC pure product (2.48 g, 82% yield). The same method was applied to prepare the rest of the other ligands **(L2)-(L6)**.

N-(4,6-dimethylpyrimidin-2-yl)-4-[(2-hydroxy-naphthalen-1-yl)methyleneamino]-benzenesulfonamide (L_1). Yield 82% (2.48 g); m.p. 240–41°C; IR (KBr, cm^{-1}): 3235 (NH), 3315 (OH), 1597 (HC═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), 7.90–8.25 (m, 6H, naphthalene), 9.15 (s, 1H, azomethine), 10.85 (s, 1H, OH), 11.34 (s, 1H, SO_2NH); ^{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), 160.0 (C═N, azomethine), 108.5 (C_1-naphthalene), 158.8 (C_2-naphthalene), 118.4 (C_3-naphthalene), 132.4 (C_4-naphthalene), 126.8–135.1 (C_5,C_6,C_7,C_8, C_9,C_{10}-naphthalene); Anal. Calcd. for $C_{23}H_{20}N_4O_3S$ (432.50): C, 63.87; H, 4.66; N, 12.95; Found: C, 63.78; H, 4.75; N, 13.01%. Mass spectrum (ESI) $[M]^+$ = 431.8. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 2.35 (s, 6H, CH_3), 6.99 (s, 1H, pyrimidine), 7.80–8.15 (m, 4H, N-Ph), 8.20–8.55 (m, 6H, naphthalene), 9.45 (s, 1H, azomethine), 11.64 (s, 1H, SO_2NH). ^{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), 114.6 (C_1-naphthalene), 170.1 (C_2-naphthalene), 122.3 (C_3-naphthalene), 126.8–135.1 (C_4-C_{10}-naphthalene).

N-(pyrimidin-2-yl)-4-[(2-hydroxynaphthalen-1-yl)methyleneamino]-benzenesulfonamide (L_2). Yield 73% (2.07 g); m.p. 260–62°C; IR (KBr, cm^{-1}): 3237 (NH), 3315 (OH), 1597 (HC═N), 1542 (-N = pyrimidine ring), 1326, 1122 (S═O), 957 (S—N), 836 (C—S); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.01–7.44 (m, 3H, pyrimidine), 7.50–7.85 (m, 4H, N-Ph), 7.90–8.25 (m, 6H, naphthalene), 9.15 (s, 1H, azomethine), 10.85 (s, 1H, OH), 11.34 (s, 1H, SO_2NH); ^{13}C NMR (δ, ppm): 157.9 (C_4,C_6-pyrimidine), 110.2 (C_5-pyrimidine), 159.3 (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), 160.0 (C═N, azomethine), 108.5 (C_1-naphthalene), 158.8 (C_2-naphthalene), 118.4 (C_3-naphthalene), 132.4 (C_4-naphthalene), 126.8–135.1 (C_5,C_6,C_7, C_8,C_9,C_{10}-naphthalene); Anal. Calcd. for $C_{21}H_{16}N_4O_3S$ (404.45): C, 62.36; H, 3.99; N, 13.85; Found: C, 62.32; H, 4.05; N, 13.80%. Mass spectrum (ESI) $[M]^+$ = 404.80. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 7.25–7.69 (m, 3H, pyrimidine), 7.80–8.15 (m, 4H, N-Ph), 8.20–8.55 (m, 6H, naphthalene), 9.45 (s, 1H, azomethine), 11.67 (s, 1H, SO_2NH); ^{13}C NMR of Zn (II) complex (δ, ppm): 157.9 (C_4,C_6-pyrimidine), 110.2 (C_5-pyrimidine), 159.3 (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), 114.6 (C_1-naphthalene), 170.1 (C_2-naphthalene), 122.3 (C_3-naphthalene), 126.8-135.1 (C_4-C_{10}-naphthalene).

N-(3,4-dimethylisoxazol-5-yl)-4-[(2-hydroxy-naphthalen-1-yl)methyleneamino]- benzenesulfonamide (L_3). Yield 81% (2.39 g); m.p. 271–72°C; IR (KBr, $cm^{-1)}$: 3233 (NH), 3315 (OH), 1597 (HC═N), 1320, 1118 (S═O), 957 (S-N), 838 (C-S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.35 (m, 6H, CH3), 7.50–7.85 (m, 4H, N-Ph), 7.90–8.25 (m, 6H, naphthalene), 9.15 (s, 1H, azomethine), 10.85 (s, 1H, OH), 11.45 (s, 1H, SO_2NH); ^{13}C NMR (δ, ppm): 15.1 (CH_3-isoxazol), 9.5 (CH_3-isoxazol), 159.9 (C_3-isoxazole), 100.5 (C_4-isoxazole), 158.9 (C_5-isoxazol), 138.2 (C_1-phenyl), 128.6 (C_2,C_6-phenyl), 122.6 (C_3,C_5-phenyl), 156.4 (C_4-phenyl), 160.0 (C═N, azomethine), 108.5 (C_1-naphthalene), 158.8 (C_2-naphthalene), 118.4 (C_3-naphthalene), 132.4 (C_4-naphthalene), 126.8–135.1 (C_5-C_{10}-naphthalene); Anal. Calcd. for $C_{22}H_{19}N_3O_4S$ (421.48): C, 62.69; H, 4.54; N, 9.97; Found: C, 62.82; H, 4.75; N, 9.86%. Mass spectrum (ESI) $[M]^+$ = 420.80. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 2.55 (m, 6H, CH_3), 7.80–8.15 (m, 4H, N-Ph), 8.20–8.55 (m, 6H, naphthalene), 9.45 (s, 1H, azomethine), 11.65 (s, 1H, SO_2NH); ^{13}C NMR of Zn (II) complex (δ, ppm): 15.1 (CH_3-isoxazol), 9.5 (CH_3-isoxazol), 159.9 (C_3-isoxazole), 100.5 (C_4-isoxazole), 158.9 (C_5-isoxazol), 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), 114.6 (C_1-naphthalene), 170.1 (C_2-naphthalene), 122.3 (C_3-naphthalene), 126.8–135.1 (C_4-C_{10}-naphthalene).

N-(5-methylisoxazol-3-yl)-4-[(2-hydroxynaphthalen-1-yl)methyleneamino]- benzenesulfonamide (L_4). Yield 76% (2.17 g); m.p. 255–56°C; IR (KBr, cm^{-1}): 3230 (NH), 3315 (OH), 1597 (HC═N), 1323, 1118 (S═O), 956 (S—N), 836 (C—S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.29 (s, 3H, CH3), 6.09 (s, 1H, isoxazol), 7.50–7.85 (m, 4H, N-Ph), 7.90–8.25 (m, 6H, naphthalene), 9.15 (s, 1H, azomethine), 10.85 (s, 1H, OH), 11.43 (s, 1H, SO_2NH); ^{13}C NMR (δ, ppm): 12.8 (CH_3-isoxazol), 159.6 (C_5-isoxazole), 95.0 (C_4-isoxazole), 150.0 (C_3-isoxazol), 138.2 (C_1-phenyl), 128.6 (C_2,C_6-phenyl), 122.6 (C_3,C_5-phenyl), 156.4 (C_4-phenyl), 160.0 (C═N, azomethine), 108.5 (C_1-naphthalene), 158.8 (C_2-naphthalene), 118.4 (C_3-naphthalene), 132.4 (C_4-naphthalene), 126.8–135.1 (C_5-C_{10}-naphthalene); Anal. Calcd. for $C_{21}H_{17}N_3O_4S$ (407.45): C, 61.91; H, 4.21; N, 10.31; Found: C, 61.82; H, 4.38; N, 10.28%. Mass spectrum (ESI) $[M]^+$ = 406.80. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 2.57 (s, 3H, CH_3), 6.42 (s, 1H, isoxazol), 7.80–8.15 (m, 4H, N-Ph), 8.20–8.55 (m, 6H, naphthalene), 9.45 (s, 1H, azomethine), 11.73 (s, 1H, SO_2NH); ^{13}C NMR of Zn (II) complex (δ, ppm): 12.8 (CH_3-isoxazol), 159.6 (C_5-isoxazole), 95.0 (C_4-isoxazole), 150.0 (C_3-isoxazol), 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), 114.6 (C_1-naphthalene), 170.1 (C_2-naphthalene), 122.3 (C_3-naphthalene), 126.8–135.1 (C_4-C_{10}-naphthalene).

N-(thiazol-2-yl)-4-[(2-hydroxynaphthalen-1-yl)methyleneamino]- benzenesulfonamide (L_5). Yield 82% (2.35 g); m.p. 281–82°C; IR (KBr, cm^{-1}): 3236 (NH), 3315 (OH), 1597 (HC═N), 1324, 1122 (S═O), 953 (S—N), 837 (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), 7.90–8.25 (m, 6H, naphthalene), 9.15 (s, 1H, azomethine), 10.85 (s, 1H, OH), 11.55 (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), 160.0 (C═N, azomethine), 108.5 (C_1-naphthalene), 158.8 (C_2-naphthalene), 118.4 (C_3-naphthalene), 132.4 (C_4-naphthalene), 126.8–135.1 (C_5-C_{10}-naphthalene); Anal. Calcd. for $C_{20}H_{15}N_3O_3S_2$ (409.49): C, 58.66; H, 3.69; N, 10.26; Found: C, 58.56; H, 3.62; N, 10.48%. Mass spectrum (ESI) $[M]^+$ = 408.80. 1H NMR of Zn (II) complex (DMSO-d6, δ, ppm): 7.01–7.32 (m, 2H, thiazol), 7.80–8.15 (m, 4H, N-Ph), 8.20–8.55 (m, 6H, naphthalene), 9.45 (s, 1H, azomethine), 11.75 (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), 114.6 (C_1-naphthalene), 170.1 (C_2-naphthalene), 122.3 (C_3-naphthalene), 126.8–135.1 (C_4-C_{10}-naphthalene).

N-carbamimidoyl-4-[(2-hydroxynaphthalen-1-yl)methyleneamino]- benzenesulfonamide (L_6). Yield 79% (2.04 g); m.p. 248–49°C; IR (KBr, cm^{-1}): 3232 (NH), 3315 (OH), 1597 (HC═N), 1325, 1120 (S═O), 951 (S—N), 833 (C—S); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.63 (m, 2H, NH_2), 7.43 (m, 1H, NH), 7.50–7.85 (m, 4H, N-Ph), 7.90–8.25 (m, 6H, naphthalene), 9.15 (s, 1H, azomethine), 10.85 (s, 1H, OH), 11.40 (s, 1H, SO_2NH); ^{13}C NMR (δ, ppm): 163.0 (C-carbamimidoyl), 138.2 (C_1-phenyl), 128.6 (C_2,C_6-phenyl), 122.6 (C_3,C_5-phenyl), 156.4 (C_4-phenyl), 160.0 (C═N, azomethine), 108.5 (C_1-naphthalene), 158.8 (C_2-naphthalene), 118.4 (C_3-naphthalene), 132.4 (C_4-naphthalene), 126.8–135.1 (C_5-C_{10}-naphthalene); Anal. Calcd. for $C_{18}H_{16}N_4O_3S$ (368.41): C, 58.68; H, 4.38; N, 15.21; Found: C, 58.75; H, 4.42; N, 15.18%. Mass spectrum (ESI) $[M]^+$ = 367.80. ^{1}H NMR of Zn (II) complex (DMSO-d6, δ, ppm): 6.83 (m, 2H, NH_2), 7.65 (m, 1H, NH), 7.80–8.15 (m, 4H, N-Ph), 8.20–8.55 (m, 6H, naphthalene), 9.45 (s, 1H, azomethine), 11.63 (s, 1H, SO_2NH); ^{13}C NMR of Zn (II) complex (δ, ppm): 163.0 (C-carbamimidoyl), 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), 114.6 (C_1-naphthalene), 170.1 (C_2-naphthalene), 122.3 (C_3-naphthalene), 126.8–135.1 (C_4-C_{10}-naphthalene).

Synthesis of metal (II) complexes

Synthesis of Co (II) Complex with N-(4,6-dimethylpyrimidin-2-yl)-4-[(2-hydroxy-naphthalen-1-yl-methyleneamino]benzenesulfonamide [Co(L_1-H)$_2$(H_2O)$_2$] (1). To a hot magnetically stirred dioxane (10 mL) solution of N-(4,6-dimethylpyrimidin-2-yl)-4-[(2-hydroxy-naphthalen-1-yl-methyleneamino]benzenesulfonamide **(L_1)** (0.87 g, 0.002 moles), an aqueous solution (15 mL) of Co (II) $Cl_2.6H_2O$ (0.24 g, 0.001 moles) was added. Ammonia-ammonium chloride buffer solution (pH = 10.0, 2 mL) was added in it to maintain the pH of the reaction mixture. The mixture was then refluxed for 1 h. The precipitates formed during refluxing, were cooled to room temperature, collected by suction filtration and washed with small amount of dioxane (1x5 mL), ether (2 × 10 mL) and dried. Unfortunately, only microcrystalline powder could be obtained, which was impossible to be used for X-ray structural determinations.

The same method was used for the preparation of all other complexes **(2)-(24).**

Biological properties

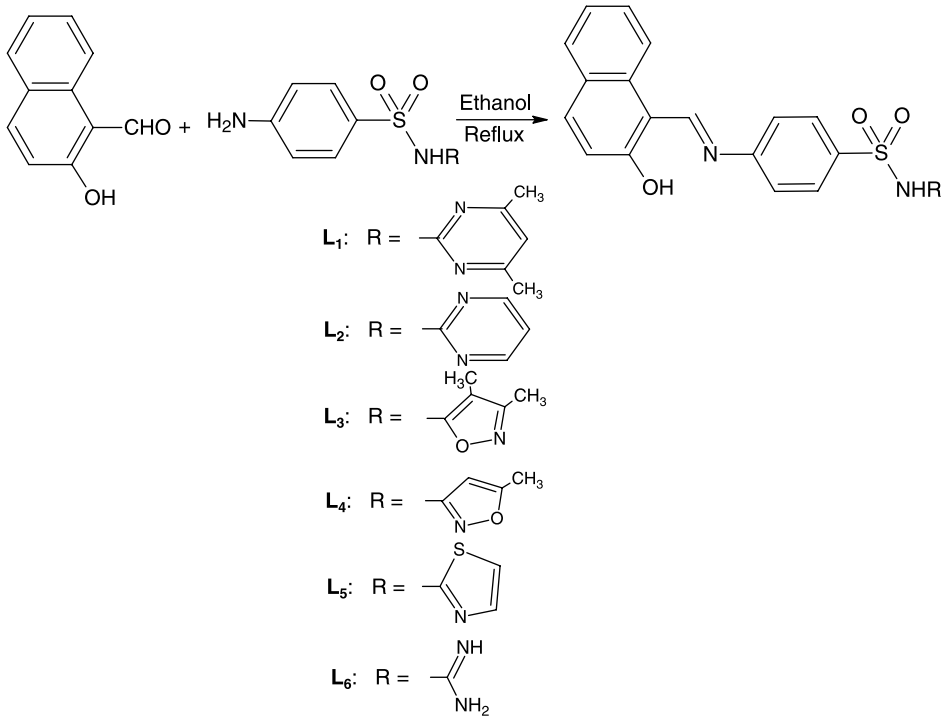
Antibacterial bioassay (in vitro). All the synthesized compounds **(L_1)-(L_6)** and metal (II) complexes

(1)-(24) were screened *in vitro* for their antibacterial activity against four Gram-negative (*Escherichia coli, Shigella flexeneri, Pseudomonas aeruginosa, Salmonella typhi*) and two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) bacterial strains by the agar-well diffusion method [25,26]. The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Two to eight hours 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, England) 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 seven days. The results were recorded [27] as % inhibition and compared with standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration (MIC). 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 [28].

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 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 DMF. From this stock solutions 500, 50 and 5 μg/mL were transferred to 9 vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and



Scheme 1. Preparation of Ligands

one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two 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 analyzed by Finney computer program to determine the LD_{50} values [29,30].

Results and discussion

Chemistry, composition and characterization of the ligands

The sulfonamide derived ligands **(L_1)-(L_6)** 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)-(24)** of the ligands **(L_1)-(L_6)** were prepared according to the following equation:

$$MCl_2 + 2\ \text{Ligand (L)} \rightarrow [M(L-H)_2(OH_2)_2]$$

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

$$L = (L_1) - (L_6)$$

Physical measurements and Analytical data for complexes **(1)-(24)** is given in Table I.

Conductance and magnetic susceptibility measurements. The molar conductance values (in DMF) for complexes **(1)**-(**24)** fall within the range 84–94 Ω^{-1} cm^2 mol^{-1}, showing their non-electrolytic [31] nature. The room temperature magnetic moment values of the complexes are given in Table II. The observed magnetic moment (4.78–4.86 B.M.) is consistent with half-spin octahedral cobalt (II) complexes. The magnetic moment values (1.77–1.85 B.M.) measured for the copper (II) complexes lie in the range expected for a d^9- system, which contain one unpaired electron with octahedral geometry [32]. The measured values (3.28–3.33 B.M.) for the nickel (II) complexes also suggest [33] 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 experimental and in Table II. All ligands contain various potential donor sites. In the IR spectra of the ligands a broad band observed at 3315 cm^{-1} and a sharp band at 1597 cm^{-1} are assigned [34] to the ν(OH) and ν(C=N) modes respectively. Evidence of the nitrogen bonding of the azomethine ν(C=N)

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

No.			M.P (dec.) (°C)	Yield (%)	Calc. (Found) %		
					C	H	N
1.	$[Co(L_1\text{-}H)_2(H_2O)_2]$ $C_{46}H_{42}N_8O_8S_2$ Co	[957.95]	270–275	80	57.68(57.12)	4.42(4.33)	11.70(11.42)
2.	$[Cu(L_1\text{-}H)_2(H_2O)_2]$ $C_{46}H_{42}N_8O_8S_2$ Cu	[962.56]	255–260	79	57.40(57.47)	4.40(4.87)	11.64(11.84)
3.	$[Ni(L_1\text{-}H)_2(H_2O)_2]$ $C_{46}H_{42}N_8O_8S_2$ Ni	[957.71]	268–273	81	57.69(57.66)	4.42(4.21)	11.70(11.32)
4.	$[Zn(L_1\text{-}H)_2(H_2O)_2]$ $C_{46}H_{42}N_8O_8S_2$ Zn	[964.41]	262–264	75	57.29(57.69)	4.39(4.46)	11.62(11.73)
5.	$[Co(L_2\text{-}H)_2(H_2O)_2]$ $C_{42}H_{34}N_8O_8S_2$ Co	[901.84]	299–304	82	55.94(55.41)	3.80(4.18)	12.42(12.65)
6.	$[Cu(L_2\text{-}H)_2(H_2O)_2]$ $C_{42}H_{34}N_8O_8S_2$ Cu	[906.46]	277–283	84	55.65(55.63)	3.78(3.81)	12.36(12.43)
7.	$[Ni(L_2\text{-}H)_2(H_2O)_2]$ $C_{42}H_{34}N_8O_8S_2$ Ni	[901.60]	283–288	81	55.95(55.85)	3.80(4.01)	12.43(12.36)
8.	$[Zn(L_2\text{-}H)_2(H_2O)_2]$ $C_{42}H_{34}N_8O_8S_2$ Zn	[908.30]	268–273	80	55.54(55.81)	3.77(3.87)	12.34(12.08)
9.	$[Co(L_3\text{-}H)_2(H_2O)_2]$ $C_{44}H_{40}N_6O_{10}S_2$ Co	[935.90]	295–298	77	56.47(56.66)	4.31(4.38)	8.98(8.84)
10.	$[Cu(L_3\text{-}H)_2(H_2O)_2]$ $C_{44}H_{40}N_6O_{10}S_2$ Cu	[940.51]	280–284	81	56.19(56.42)	4.29(4.37)	8.94(8.98)
11.	$[Ni(L_3\text{-}H)_2(H_2O)_2]$ $C_{44}H_{40}N_6O_{10}S_2$ Ni	[935.66]	290–295	82	56.48(56.67)	4.31(4.52)	8.98(8.78)
12.	$[Zn(L_3\text{-}H)_2(H_2O)_2]$ $C_{44}H_{40}N_6O_{10}S_2$ Zn	[942.36]	297–299	79	56.08(56.16)	4.28(4.32)	8.92(8.82)
13.	$[Co(L_4\text{-}H)_2(H_2O)_2]$ $C_{42}H_{36}N_6O_{10}S_2$ Co	[907.84]	245–250	83	55.57(55.46)	4.00(4.08)	9.26(9.24)
14.	$[Cu(L_4\text{-}H)_2(H_2O)_2]$ $C_{42}H_{36}N_6O_{10}S_2$ Cu	[912.46]	265–270	81	55.29(55.42)	3.98(4.07)	9.21(9.28)
15.	$[Ni(L_4\text{-}H)_2(H_2O)_2]$ $C_{42}H_{36}N_6O_{10}S_2$ Ni	[907.60]	256–261	82	55.58(55.67)	4.00 (4.12)	9.26 (9.28)
16.	$[Zn(L_4\text{-}H)_2(H_2O)_2]$ $C_{42}H_{36}N_6O_{10}S_2$ Zn	[914.30]	277–280	75	55.17(55.36)	3.97(4.12)	9.19(9.22)
17.	$[Co(L_5\text{-}H)_2(H_2O)_2]$ $C_{40}H_{32}N_6O_8S_4$ Co	[911.92]	288–294	83	52.68(52.56)	3.54(3.58)	9.22(9.24)
18.	$[Cu(L_5\text{-}H)_2(H_2O)_2]$ $C_{40}H_{32}N_6O_8S_4$ Cu	[916.54]	272–276	81	52.42(52.48)	3.52(3.67)	9.17(9.28)
19.	$[Ni(L_5\text{-}H)_2(H_2O)_2]$ $C_{40}H_{32}N_6O_8S_4$ Ni	[911.68]	288–292	82	52.70(52.67)	3.54(3.52)	9.22(9.28)
20.	$[Zn(L_5\text{-}H)_2(H_2O)_2]$ $C_{40}H_{32}N_6O_8S_4$ Zn	[918.38]	267–273	73	52.31(52.46)	3.51(3.52)	9.15(9.22)
21.	$[Co(L_6\text{-}H)_2(H_2O)_2]$ $C_{36}H_{34}N_8O_8S_2$ Co	[829.78]	260–65	78	52.11(52.18)	4.13(4.38)	13.50(13.44)
22.	$[Cu(L_6\text{-}H)_2(H_2O)_2]$ $C_{36}H_{34}N_8O_8S_2$ Cu	[834.39]	250–254	79	51.82(51.62)	4.11(4.27)	13.43(13.68)
23.	$[Ni(L_6\text{-}H)_2(H_2O)_2]$ $C_{36}H_{34}N_8O_8S_2$ Ni	[829.53]	255–260	77	52.13(52.17)	4.13(4.32)	13.51(13.58)
24.	$[Zn(L_6\text{-}H)_2(H_2O)_2]$ $C_{36}H_{34}N_8O_8S_2$ Zn	[836.23]	263–268	74	51.71(51.96)	4.10(4.22)	13.40(13.22)

Table II. Conductivity, magnetic and spectral data of the metal (II) complexes.

No.	Ω_M (Ω^{-1} cm^2 mol^{-1})	B.M (μ_{eff})	λ_{max} (cm^{-1})	IR (cm^{-1})
1.	86.2	4.82	7290,17430, 20490,29325	1577 (C═N), 1395 (C—O), 1325, 1120 (SO_2), 955 (S—N), 835 (C—S), 440 (M—N), 525 (M—O)
2.	87.5	1.77	14845,19165, 30355	1567 (C═N), 1395 (C—O), 1325, 1120 (SO_2), 955 (S—N), 835 (C—S), 435 (M—N), 520 (M—O)
3.	86.8	3.31	10445,15725, 26440,29975	1570 (C═N), 1395 (C—O), 1325, 1120 (SO_2), 955 (S—N), 835 (C—S), 437 (M—N), 545 (M—O)
4.	92.3	Dia	28630	1568 (C═N), 1395 (C—O), 1325, 1120 (SO_2), 955 (S—N), 835 (C—S), 437 (M—N), 528 (M—O)
5.	93.7	4.86	7470,17520, 20680,29385	1569 (C═N), 1395 (C—O), 1326, 1122 (SO_2), 957 (S—N), 836 (C—S), 444 (M—N), 525 (M—O)
6.	93.2	1.78	15160,19315, 30380	1577 (C═N), 1395 (C—O), 1326, 1122 (SO_2), 957 (S—N), 836 (C—S), 442 (M—N), 520 (M—O)
7.	94.0	3.30	10490,15865, 26675,30225	1570 (C═N), 1395 (C—O), 1326, 1122 (SO_2), 957 (S—N), 836 (C—S), 438 (M—N), 533 (M—O)
8.	86.3	Dia	29145	1568 (C═N), 1395 (C—O), 1326, 1122 (SO_2), 957 (S—N), 836 (C—S), 436 (M—N), 545 (M—O)
9.	84.8	4.78	7275,17355, 20445,29280	1570 (C═N), 1395 (C—O), 1320, 1118 (SO_2), 957 (S—N), 838 (C—S), 435 (M—N), 530 (M—O)
10.	84.0	1.85	14720,19140, 30335	1567 (C═N), 1395 (C—O), 1320, 1118 (SO_2), 957 (S—N), 838 (C—S), 443 (M—N), 535 (M—O)
11.	85.2	3.31	10490,15610, 26325,29850	1570 (C═N), 1395 (C—O), 1320, 1118 (SO_2), 957 (S—N), 838 (C—S), 440 (M—N), 545 (M—O)
12.	86.4	Dia	28530	1568 (C═N), 1395 (C—O), 1320, 1118 (SO_2), 957 (S—N), 838 (C—S), 439 (M—N), 523 (M—O)
13.	87.7	4.79	7285,17360, 20495,29395	1573 (C═N), 1395 (C—O), 1323, 1118 (SO_2), 956 (S—N), 836 (C—S), 435 (M—N), 536 (M—O)
14.	89.2	1.81	14765,19140, 30335	1575 (C═N), 1395 (C—O), 1323, 1118 (SO_2), 956 (S—N), 836 (C—S), 439 (M—N), 540 (M—O)
15.	88.3	3.33	10350,15610, 26325,29850	1570 (C═N), 1395 (C—O), 1323, 1118 (SO_2), 956 (S—N), 836 (C—S), 435 (M—N), 535 (M—O)
16.	87.6	Dia	28780	1568 (C═N), 1395 (C—O), 1323, 1118 (SO_2), 956 (S—N), 836 (C—S), 445 (M—N), 523 (M—O)
17.	90.5	4.84	7485,17360,20555,29285	1575 (C═N), 1395 (C—O), 1324, 1122 (SO_2), 953 (S—N), 837 (C—S), 439 (M—N), 538 (M—O)
18.	92.3	1.83	14775,19140,30335	1577 (C═N), 1395 (C—O), 1324, 1122 (SO_2), 953 (S—N), 837 (C—S), 437 (M—N), 527 (M—O)
19.	93.4	3.29	10355,15610,26325,29850	1570 (C═N), 1395 (C—O), 1324, 1122 (SO_2), 953 (S—N), 837 (C—S), 440 (M—N), 535 (M—O)
20.	88.4	Dia	28990	1568 (C═N), 1395 (C—O), 1324, 1122 (SO_2), 953 (S—N), 837, (C—S), 439 (M—N), 523 (M—O)
21.	93.9	4.80	7285,17360,20475,29360	1569 (C═N), 1395 (C—O), 1325, 1120 (SO_2), 951 (S—N), 833 (C—S), 445 (M—N), 530 (M—O)
22.	91.0	1.85	15160,19140,30335	1571 (C═N), 1395 (C—O), 1325, 1120 (SO_2), 951 (S—N), 833 (C—S), 435 (M—N), 520 (M—O)
23.	86.3	3.28	10355,15610,26325,29850	1570 (C═N), 1395 (C—O), 1325, 1120 (SO_2), 951 (S—N), 833 (C—S), 440 (M—N), 535 (M—O)
24.	87.4	Dia	28755	1568 (C═N), 1395 (C—O), 1325, 1120 (SO_2), 951 (S—N), 833 (C—S), 439 (M—N), 523 (M—O)

group to the central metal atom stems from the shift of the ν(C═N) frequency to lower frequency side by 20–30 cm^{-1} (1567–1577 cm^{-1}) in all of its metal complexes. This is further supported by the appearance of the new bands at 435–445 cm^{-1} assignable to the ν(M—N) [35].

The coordination through the hydroxyl oxygen is revealed by the disappearance of the mode at 3315 cm^{-1} and appearance of a new band at 1395 cm^{-1} due to the C—O mode. This is further confirmed by the appearance of the new band at 520–545 cm^{-1} due to ν(M—O) in the metal complexes. The bands in the ligand due to $\nu_{asymm}(SO_2)$ and $\nu_{symm}(SO_2)$ appear at 1320–1326 and 1118–1122 cm^{-1}, respectively [36]. These bands remain almost unchanged in the complexes, indicating that this group is not involved in coordination. This is supported by the unchanged ν(S—N) and ν(C—S) modes appearing at 951–957 and 833–838 cm^{-1}, respectively [37,38], in the ligands after complexation. All the other potential electron pair donor sites of the ligands do not participate in coordination as their IR frequencies remain unchanged after complexation.

^{1}H NMR Spectra. ^{1}H NMR spectra of the free ligands and their diamagnetic zinc (II) complexes were recorded in DMSO-d_6. The ^{1}H NMR spectral data along with the possible assignments is recorded in the Experimental. All the protons due to heteroaromatic/aromatic groups were found as to be in their expected region [39]. 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 9.15 ppm in the ligand to 9.45 ppm in the complexes. Hydroxyl proton at 10.85 ppm in the spectra of Zn (II) complexes of ligands **(L_1)-(L_6)** disappeared indicating deprotona-tion and coordination of the oxygen with the metal ion All other protons underwent downfield shifting by 0.25–0.45 ppm due to the increased conjugation [40] 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 is recorded in the experimental part. 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 160.0 ppm in the ligand to 173.3 ppm in its metal (II) complexes revealed coordination of the azomethine nitrogen to the metal atom. All other carbons underwent a downfield shift by 4.0–12.0 ppm due to the increased conjugation and coordination with the metal atoms. Furthermore, the the number of carbons present agreed well with the expected values.

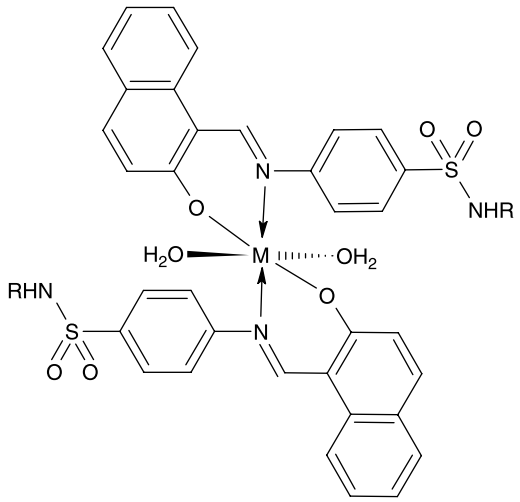
Mass spectra. The mass spectral data is consistent with the formulations: $C_{23}H_{20}N_4O_3S$, 431.8 (calcd., 432.50); $C_{21}H_{16}N_4O_3S$, 404.80 (calcd., 404.45); $C_{22}H_{19}N_3O_4S$, 420.80 (calcd., 421.48); $C_{21}H_{17}N_3O_4S$, 406.80 (calcd., 407.45); $C_{20}H_{15}N_3O_3S_2$, 408.80 (calcd., 409.49); $C_{18}H_{16}N_4O_3S$, 367.80 (calcd., 368.41) of the ligands. The base peak for $[C_{17}H_{12}NO]^+$ was observed at 245.9 for all the ligands as it is expected to be the most stable fragment.

Electronic spectra. The Co(II) complexes exhibited well-resolved, low-energy bands at 7,275–7,485 cm^{-1}, 17,355–17,520 cm^{-1} and a strong high-energy band at 20,445–20,680 cm^{-1} (Table–II) which are assigned [32] 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 [33]. 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 [41] consistency with their octahedral environment.

The electronic spectra of the Cu (II) complexes (Table–II) showed two low-energy weak bands at 14,720–15,160 cm^{-1} 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 [42]. 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 [33].

The electronic spectra of the Ni (II) complexes (Table–II) showed d-d bands in the region 10,350–10,490, 15,610–15,865 and 26,325–26,675 cm^{-1}. These are assigned [41] 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 [42] also an octahedral geometry for the Ni (II) complexes. The electronic spectra of the Zn (II) complexes

(Table–II) exhibited only a high-intensity band at 28,530–29,145 cm^{-1} and are assigned [43] to a ligand-metal charge transfer.



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

Scheme 2. Proposed Structure of the Metal (II) Complexes

Biological activity

Antibacterial bioassay (in vitro). All compounds were tested against four Gram-negative (*E. coli*, *S. flexeneri*, *P. aeruginosa*, *S. typhi*) and two Gram-positive (*S. aureus*, *B. subtilis*) bacterial strains (Table III) according to the literature protocol [26,27]. The results were compared with those of the standard drug imipenum (Figure 1). All ligands showed moderate to significant activity against all Gram-negative and Gram-positive bacterial strains except the activity of all compounds against *S. flexeneri* where no moderate to significant activity was observed. Compounds **(1)-(24)** exhibited overall a significant activity against *E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus* and *B. subtilis*. However, the compound **(24)** was the most active one. Antibacterial activity is overall enhanced after complexation of the ligands (Figure 2). However the Zinc (II) complexes of all the ligands were observed to be the most active against all species.

Antifungal bioassay (in vitro). The antifungal screening of all compounds was carried out against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*,

Table III. Antibacterial Bioassay of Ligands and Metal (II) Complexes*.

Bacteria	Compound [zone of inhibition (mm)]																													
	L_1	L_2	L_3	L_4	L_5	L_6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	SD
Gram-negative																															
(a)	16	15	17	12	14	13	19	12	20	24	19	21	22	21	19	20	19	23	15	16	15	20	17	15	21	24	19	20	19	25	30
(b)	08	08	06	07	09	08	08	06	10	10	11	08	09	10	12	10	11	12	11	12	13	13	11	11	10	12	11	11	10	14	27
(c)	14	13	15	13	15	15	18	19	18	21	17	16	20	20	18	19	20	24	17	18	16	21	18	19	20	22	18	19	20	23	26
(d)	12	13	13	14	15	14	20	17	18	22	18	18	19	20	20	19	20	21	18	18	18	20	20	20	19	23	20	17	18	24	27
Gram-positive																															
(e)	17	17	16	16	17	16	17	16	17	23	20	21	19	21	19	20	20	22	17	18	17	24	19	19	19	24	19	20	18	26	30
(f)	15	16	19	15	14	15	20	18	15	18	17	21	17	22	20	19	22	24	18	17	18	22	19	19	18	23	20	19	22	24	28

(a) = E. coli, **(b)** = S. flexenari, **(c)** = P. aeruginosa, **(d)** = S. typhi, **(e)** = S. aureus, **(f)** = B. subtilis
< 10: weak; >10: moderate; > 16: Significant. SD = Standard Drug (Imipenum)

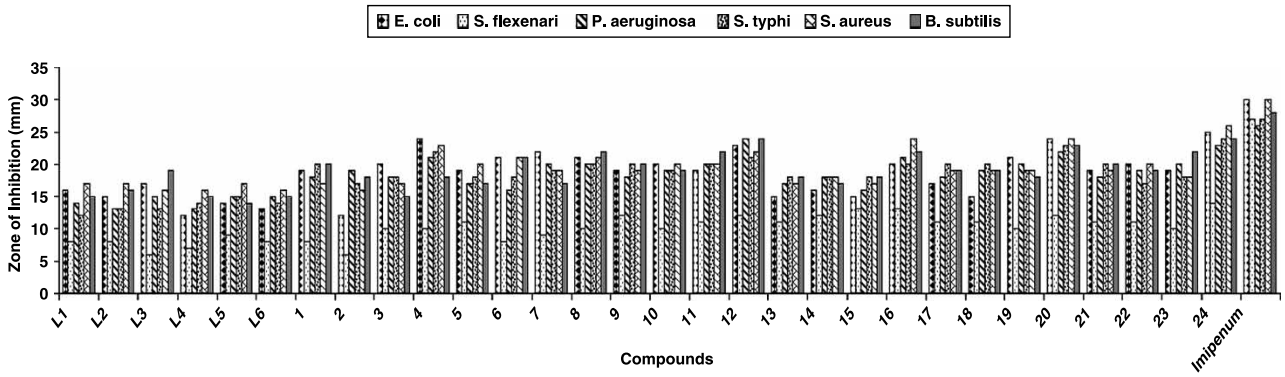


Figure 1. Comparison of antibacterial activity.

*concentration used 1 mg/mL of DMSO

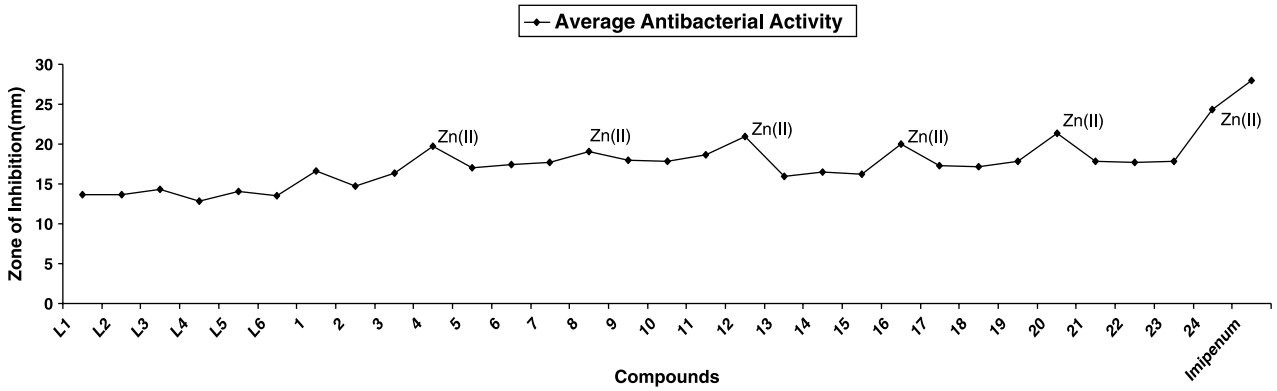


Figure 2. Average antibacterial activity of ligands versus metal (II) complexes.

Table IV. Antifungal Bioassay of Ligands and Metal (II) Complexes⋆.

Organism	Compound (% inhibition)																													
	L_1	L_2	L_3	L_4	L_5	L_6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	SD
(a)	70	58	40	80	00	80	35	50	00	00	50	60	40	00	80	40	00	80	55	35	40	20	45	65	00	00	78	30	45	25	A
(b)	55	00	00	00	65	00	40	00	35	70	50	00	60	00	00	30	70	55	75	45	35	60	40	00	00	75	65	60	65	60	B
(c)	80	00	70	80	00	20	10	30	00	00	60	80	00	40	00	00	00	40	65	00	55	55	35	40	40	40	45	45	60	50	C
(d)	60	75	75	80	00	80	00	00	70	50	55	55	00	70	35	35	20	80	00	60	00	00	90	35	85	85	00	00	35	55	D
(e)	00	52	90	20	20	65	00	40	80	00	40	45	70	90	45	60	20	00	00	90	00	70	00	50	30	00	00	20	80	85	E
(f)	50	30	00	00	40	00	20	00	80	70	00	60	50	00	55	00	75	00	80	35	00	80	60	00	00	00	70	00	40	40	F

(a) = T. longifucus, **(b)** = C. Albicans, **(c)** = A. flavus, **(d)** = M. canis, **(e)** = F. Solani, **(f)** = C. glaberata
⋆(concentration used 200 μg/mL)
SD = Standard Drugs MIC μg/mL; A = Miconazole (70 μg/mL: 1.6822×10^{-4} M),B = Miconazole (110.8 μg/mL:2.6626×10^{-4} M), C = Amphotericin B (20 μg/mL: 2.1642×10^{-5} M), D = Miconazole (98.4 μg/mL: 2.3647×10^{-4} M), E = Miconazole (73.25 μg/mL: 1.7603×10^{-4} M), F = Miconazole (110.8 μg/mL: 2.66266×10^{-4} M).

Microsporum canis, *Fusarium solani* and *Candida glaberata* fungal strains (Table–IV) according to the literature protocol [27]. All synthesized compounds showed good antifungal activity against different fungal strains. Compound **(23)** and **(24)** showed good antifungal activity against all the fungal strains. The results of inhibition were compared with the results of inhibition of standard drugs miconazole and amphotericin B and individual synthesized compounds were also compared (Figure 3). Effect of metal complexation on antifungal activity of the ligands can be seen (Figure 4**)**.

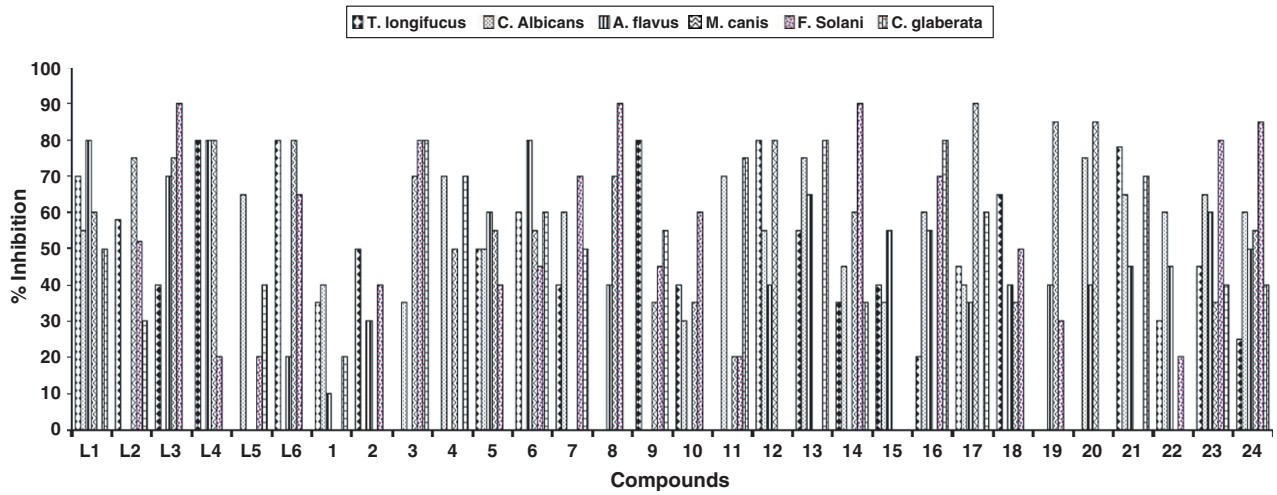


Figure 3. Comparison of antifungal activity.

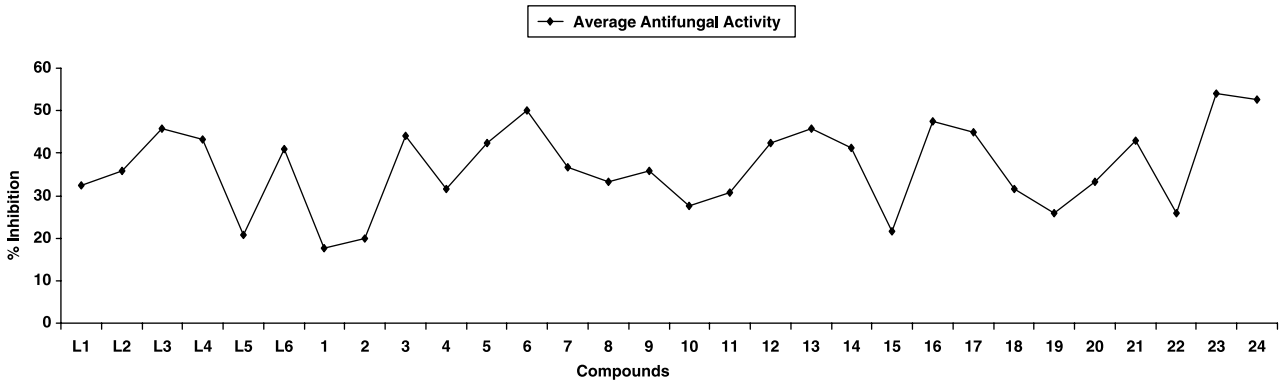


Figure 4. Average antifungal activity of ligands versus metal (II) complexes.

Table V. Minimum Inhibitory Concentration (M) of the Selected Compounds (4), (12), (16), (20 and (24) against Selected Bacteria.

No.	4	12	16	20	24
Gram-negative					
E. coli	5.185×10^{-5}	–	–	5.444×10^{-5}	5.979×10^{-5}
P. aeruginosa	1.037×10^{-4}	2.653×10^{-5}	1.094×10^{-4}	1.089×10^{-5}	2.990×10^{-5}
S. typhi	2.592×10^{-5}	–	–	2.722×10^{-5}	1.196×10^{-5}
Gram – positive					
S. aureus	–	–	5.469×10^{-5}	1.089×10^{-4}	1.196×10^{-4}
B. subtilis	–	5.306×10^{-5}	–	5.444×10^{-5}	5.979×10^{-5}

Minimum inhibitory concentration (MIC) for antibacterial activity. The preliminary antibacterial screening showed that compounds **(4), (12), (16), (20)** and **(24)** were the most active ones (above 80%). These compounds were therefore, selected for antibacterial minimum inhibitory concentration (MIC) studies (Table V).

Cytotoxic bioassay (in vitro). All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al. al* [29]. From the data recorded in Table VI, it is evident that four compounds, **(6), (10), (18)** and **(22)** displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive for this assay. The compound **(6)** showed activity ($LD_{50} = 0.601$ M), compound **(10)** showed activity ($LD_{50} = 0.484$ M), compound **(18)** showed activity ($LD_{50} = 0.606$ M), compound **(22)** showed activity ($LD_{50} = 0.627$ M) in 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 **(L_1)-(L_6)** upon chelation is rationalized on the basis of their structures and the mode of

Table VI. Brine Shrimp Bioassay Data of the Ligands (L_1)-(L_6) and their Metal (II) Complexes (1)-(24).

Compound	LD_{50} (M)
L_1	> 2.312
L_2	> 2.472
L_3	> 2.373
L_4	> 2.454
L_5	> 2.442
L_6	> 2.714
1	> 1.044
2	> 1.039
3	> 1.044
4	> 1.037
5	> 1.109
6	0.601
7	> 1.109
8	> 1.101
9	> 1.068
10	0.484
11	> 1.069
12	> 1.061
13	> 1.102
14	> 1.096
15	> 1.102
16	> 1.094
17	> 1.096
18	0.606
19	> 1.097
20	> 1.089
21	> 1.205
22	0.627
23	> 1.206
24	> 1.196

coordination/chelation. It has been suggested that chelation reduces the polarity of the metal ion [44–48] 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 [49–52] 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 [53–57] 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.

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