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Metal-based sulfonamides: synthesis, characterization, antibacterial, antifungal and cytotoxic properties of pyrrolyl- and thienyl-derived compounds

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Pyrrolyl and thienyl derived sulfonamides and their metal [cobalt(II), copper(II), nickel(II) and zinc(II)] complexes were synthesized and characterized by elemental analyses, molar conductances, magnetic moments, IR, ^{1}H NMR, ^{13}C NMR and electronic spectral data. These compounds were screened for *in-vitro* antibacterial activity against four Gram-negative (*Escherichia coli, Shigella flexeneri, Pseudomonas aeruginosa* and *Salmonella typhi*) and two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains, and for *in-vitro* antifungal activity against *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata*. The results of these studies revealed that all compounds showed significant to moderate antibacterial activity; however, the zinc complexes were shown to be the most active against various species. The brine shrimp bioassay was also carried out to study their *in vitro* cytotoxic properties of all the synthesized ligands and their metal complexes. Only two compounds (14 and 19) displayed potent cytotoxic activity as $LD_{50} = 5.5637 \times 10^{-4}$ and 4.4023×10^{-4} M ml^{-1} respectively, against *Artemia salina*. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: sulfonamides; metal complexes; antibacterial; antifungal; cytotoxicity

INTRODUCTION

The interest in metal based sulfonamides was stimulated by the successful introduction and preparation of Ag(II) and Zn(II) sulfadiazine complexes to prevent various bacterial infections.[1,2] The metal complexes of biologically active drugs/compounds work through slow release of the metal ions,[3] which is dependent on the binding nature of the complex. It is therefore important to understand the coordination environment around the metal which, in turn, is relevant to the biological activity. We began a program to prepare various metal based sulfonamides with the aim of relating the therapeutic potential of sulfonamides to the metals. We initiated this investigation with the study of different sulfonamides and *N*-substituted sulfonamides[4–6] incorporated into isatin,[7] furanyl[8] and hydroxycoumarin.[9] These compounds were potentially explored against a number of bacterial and fungal strains. Paralleling the same idea, in this paper, new thienyl and pyrrolyl derived sulfonamides and their cobalt(II), copper(II), nickel(II) and zinc(II) complexes have been prepared, characterized (elemental analyses, molar conductances, magnetic moments, IR, NMR and electronic spectral data) and evaluated for their *in vitro* antibacterial activity against four Gram-negative (*Escherichia coli, Shigella flexeneri, Pseudomonas aeruginosa* and *Salmonella typhi*) and two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains, and *in vitro* antifungal activity against *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata.* The brine shrimp bioassay was carried out as well to study their *in vitro* cytotoxic properties.

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EXPERIMENTAL

Materials and methods

Solvents used were of analytical grade; all metal (II) compounds were used as chloride salts. IR spectra were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer.

NMR spectra were recorded on a Perkin–Elmer 283B spectrometer. UV–visible spectra were obtained in DMF 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. Melting points were recorded on a Gallenkamp apparatus.

*Preparation of N-(4,6-dimethylpyrimidin-2-yl)-4-[(pyrrol-2-ylmethylene)amino]-benzenesulfonamide (**L_1**)*

To a stirred solution of the sulfamethazine (0.005 mol, 1.39 g) in ethanol (30 ml) was added a solution of pyrole-2-carboxaldehyde (0.005 mol, 0.48 g) in ethanol (15 ml). The mixture was refluxed for 3 h. The precipitates formed during refluxing were cooled to room temperature and collected by suction filtration. Washing thoroughly with ethanol afforded TLC pure products in good yield (1.33 g, 75%). All other compounds (**L_2**—**L_5**) were prepared following the same method using respectively sulfonamide and aldehyde.

*N-(4,6-dimethylpyrimidin-2-yl)-4-[(pyrrol-2-ylmethylene)amino]-benzenesulfonamide (**L_1**)*

Yield: 75% (1.33 g); m.p. 218–20 °C; IR (KBr, cm^{-1}): 3234 (NH), 1597 (azomethine, HC═N), 1555 (–N═pyrimidine ring), 1392 (C–N), 1330, 1145 (S═O), 965 (S–N), 848 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.88 (s, 6H, CH_3), 6.44–6.86 (m, 3H, pyrrolyl), 7.72 (s, 1H, azomethine), 7.64–7.70 (m, 4H, N-Ph), 8.30–8.51 (m, 1H, pyrimidine), 11.70 (s, 1H, SO_2HN); ^{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), 135.5 (C_2-pyrrol), 100.4 (C_3-pyrrol), 98.3 (C_4-pyrrol), 130.6 (C_5-pyrrol); anal. calcd for $C_{17}H_{17}N_5O_2S$ (355.42): C, 57.45; H, 4.82; N, 19.70. Found: C, 57.55; H, 4.77; N, 19.65%. ^{1}H NMR of Zn(II) complex (DMSO-d_6, δ, ppm): 3.22 (s, 6H, CH_3), 7.41–7.57 (m, 3H, pyrrolyl), 7.90–7.96 (m, 4H, N-Ph), 8.56 (s, 1H, azomethine), 8.92–9.33 (m, 1H, pyrimidine), 11.94 (s, 1H, SO_2HN); ^{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), 143.5 (C_2-pyrrol), 100.4 (C_3-pyrrol), 98.3 (C_4-pyrrol), 135.6 (C_5-pyrrol).

*N-(3,4-dimethylisoxazol-5-yl)-4-[(pyrrol-2-ylmethylene)amino]-benzenesulfonamide (**L_2**)*

Yield 68% (1.17 g); m.p. 214–15 °C; IR (KBr, cm^{-1}): 3231 (NH), 1595 (azomethine, HC═N), 1390 (C–N), 1332, 1143 (S═O), 968 (S–N), 846 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.52 (s, 6H, CH_3), 6.43–6.87 (m, 3H, pyrrolyl), 7.73 (s, 1H, azomethine), 7.65–7.72 (m, 4H, N-Ph), 11.56 (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), 135.5 (C_2-pyrrol), 100.4 (C_3-pyrrol), 98.3 (C_4-pyrrol), 130.6 (C_5-pyrrol); anal. calcd for $C_{16}H_{16}N_4O_3S$ (344.39): C, 55.80; H, 4.68; N, 16.27. Found: C, 55.82; H, 4.75; N, 16.23%. ^{1}H NMR of Zn(II) complex (DMSO-d_6, δ, ppm): 3.12 (s, 6H, CH_3), 7.55–7.61 (m, 3H, pyrrolyl), 7.91–7.97 (m, 4H, N-Ph), 8.55 (s, 1H, azomethine), 11.77 (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), 143.5 (C_2-pyrrol), 100.4 (C_3-pyrrol), 98.3 (C_4-pyrrol), 135.6 (C_5-pyrrol).

*N-pyrimidin-2-yl-4-[(2-thienylmethylene)amino]benzenesulfonamide (**L_3**)*

Yield 72% (1.24 g); m.p. 264–66 °C; IR (KBr, cm^{-1}): 3233 (NH), 1596 (azomethine, HC═N), 1550 (–N═pyrimidine ring), 1395 (C–S), 1329, 1144 (S═O), 967 (S–N), 850 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.40–6.84 (m, 3H, thienyl), 7.69 (s, 1H, azomethine), 7.59–7.66 (m, 4H, N-Ph), 8.25–8.43 (m, 3H, pyrimidine), 11.65 (s, 1H, SO_2HN); ^{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), 134.3 (C_2-thiophene), 100.4 (C_3-thiophene), 98.3 (C_4-thiophene), 129.7 (C_5-thiophene); anal. calcd for $C_{15}H_{12}N_4O_2S_2$ (344.42): C, 52.31; H, 3.51; N, 16.27. Found: C, 52.47; H, 3.65; N, 16.24%. ^{1}H NMR of Zn(II) complex (DMSO-d_6, δ, ppm): 7.45–7.58 (m, 3H, thienyl), 7.93–7.98 (m, 4H, N-Ph), 8.49 (s, 1H, azomethine), 8.97–9.35 (m, 3H, pyrimidine), 11.87 (s, 1H, SO_2HN); ^{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), 142.3 (C_2-thiophene), 100.4 (C_3-thiophene), 98.3 (C_4-thiophene), 134.7 (C_5-thiophene).

*N-(3,4-dimethylisoxazol-5-yl)-4-[(2-thienylmethylene)amino]benzenesulfonamide (**L_4**)*

Yield 73% (1.32 g); m.p: 225–27 °C; IR (KBr, cm^{-1}): 3230 (NH), 1592 (azomethine, HC═N), 1395 (C–S), 1330, 1145 (S═O), 968 (S–N), 846 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.50 (s, 6H, CH_3), 6.47–6.87 (m, 3H, pyrrolyl), 7.76 (s, 1H, azomethine), 7.59–7.70 (m, 4H, N-Ph), 11.53 (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), 134.3 (C_2-thiophene), 100.4 (C_3-thiophene), 98.3 (C_4-thiophene), 129.7 (C_5-thiophene); anal. calcd for $C_{16}H_{15}N_3O_3S_2$ (361.44): C, 53.17; H, 4.18; N, 11.63. Found: C, 53.12; H, 4.25; N, 11.58%. ^{1}H NMR of Zn(II) complex (DMSO-d_6, δ, ppm): 3.17 (s, 6H, CH_3), 7.53–7.62 (m, 3H, pyrrolyl), 7.93–7.99 (m, 4H, N-Ph), 8.57 (s, 1H, azomethine), 11.82 (s, 1H,

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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), 142.3 (C_2-thiophene), 100.4 (C_3-thiophene), 98.3 (C_4-thiophene), 134.7 (C_5-thiophene).

N-(4,6-dimethylpyrimidin-2-yl)-4-[(2-thienylmethylene)amino]-benzenesulfonamide (***L*****$_5$**)

Yield 80% (1.49 g); m.p: 220–22 °C. IR (KBr, cm^{-1}): 3240 (NH), 1594 (azomethine, HC═N), 1549 (–N═pyrimidine ring), 1397 (C–S), 1333, 1147 (S═O), 965 (S–N), 847 (C–S); ^1H NMR (DMSO-d_6, δ, ppm): 2.87 (s, 6H, CH_3), 6.42–6.85 (m, 3H, pyrrolyl), 7.71 (s, 1H, azomethine), 7.61–7.68 (m, 4H, N-Ph), 8.28–8.48 (m, 1H, pyrimidine), 11.71 (s, 1H, SO_2HN); ^{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), 134.3 (C_2-thiophene), 100.4 (C_3-thiophene), 98.3 (C_4-thiophene), 129.7 (C_5-thiophene); anal. calcd for $C_{17}H_{16}N_4O_2S_2$ (372.47): C, 54.82; H, 4.33; N, 15.04. Found: C, 54.65; H, 4.37; N, 15.08%. ^1H NMR of Zn(II) complex (DMSO-d_6, δ, ppm): 3.20 (s, 6H, CH_3), 7.40–7.55 (m, 3H, pyrrolyl), 7.91–7.95 (m, 4H, N-Ph), 8.58 (s, 1H, azomethine), 8.92–9.31 (m, 1H, pyrimidine), 11.92 (s, 1H, SO_2HN); ^{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), 142.3 (C_2-thiophene), 100.4 (C_3-thiophene), 98.3 (C_4-thiophene), 134.7 (C_5-thiophene).

*Preparation of Cobalt (II) Complex with N-(4,6-dimethylpyrimidin-2-yl)-4-[(pyrrol-2-ylmethylene) amino]benzenesulfonamide [Co $(L_2)_2(Cl)_2$] (**1**)*

To a hot magnetically stirred dioxane (20 ml) solution of *N*-(4,6-dimethylpyrimidin-2-yl)-4-[(pyrrol-2-ylmethylene) amino]benzenesulfonamide (0.002 mol, 0.71 g), an aqueous solution of the corresponding cobalt (II) chloride (0.001 mol, 0.24 g) was added. The mixture was refluxed for 2 h. The obtained solution was filtered and reduced to half of its volume by evaporation of the solvent *in vacuo*. The concentrated solution was left overnight at room temperature, which led to the formation of a solid product. It was filtered, washed with small amount of dioxane then with ether and dried. Recrystallization from 50% aqueous dioxane gave the desired products (0.60 g, 71%). All other complexes **(2–20)** were prepared following the same method using the respective metal salts as chloride respectively with different sulfonamides. Unfortunately only microcrystalline powders could be obtained, which could not be used for X-ray structural determinations.

Biological activity

*Antibacterial bioassay (*in vitro*)*

All the synthesized ligands (**L_1—L_5**) and their corresponding metal(II) complexes (**1–20**) 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 (*B. subtilis* and *S. aureus*) bacterial strains using the agar well diffusion method.[10] Two- to eight-hour-old bacterial inocula containing approximately 10^4–10^6 colony forming units (cfu) ml^{-1} were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centres at least 24 mm. The recommended concentration (100 µl) of the test sample (1 mg ml^{-1} in DMSO) was introduced into the respective wells. Other wells supplemented with DMSO and the reference antibacterial drug, imipenum, served as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared[11] with the standard drug imipenum. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions of DMSO alone and they showed no activity against any bacterial strains.

*Antifungal activity (*in vitro*)*

Antifungal activities of all compounds were studied against six fungal cultures, *T. longifusus, C. albicans, A. flavus, M. canis, F. solani* and *C. glaberata.* Sabouraud dextrose agar (Oxoid, Hampshire, UK) was seeded with 10^5 cfu ml^{-1} fungal spore suspensions and transferred to Petri plates. Disks soaked in 20 ml (200 µg ml^{-1} 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 as zones of inhibition in millimeters and compared with standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration

Compounds containing antibacterial activity over 80% were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion technique[12] by preparing disks containing 10, 25, 50 and 100 µg ml^{-1} of the compounds and applying the protocol.

*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[13] 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^{-1} were transferred to nine vials (three for each dilution were used for each test sample and LD_{50} is the mean of the three values) and one vial was

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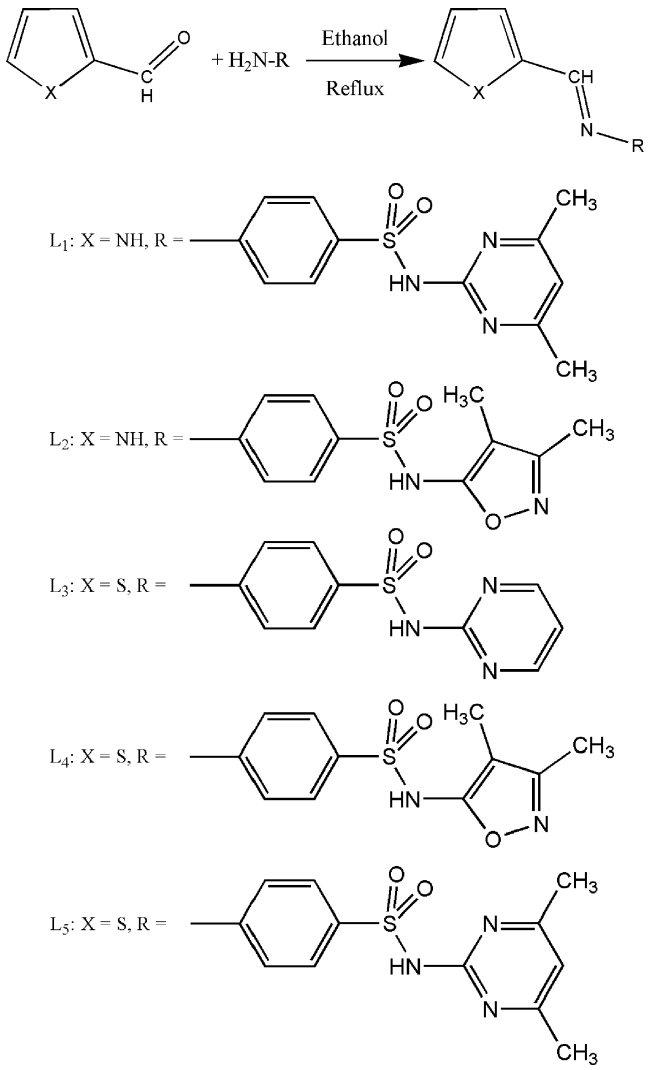
kept as control having 2 ml of DMF only. The solvent was allowed to evaporate overnight. After 2 days, when shrimp larvae were ready, 1 ml of seawater and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with seawater to 5 ml per vial. After 24 h the numbers of survivors were counted. Data were analyzed using the Finney computer program to determine the LD_{50} values.[14]

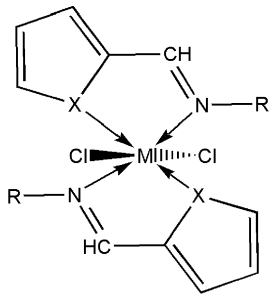
RESULTS AND DISCUSSION

Chemistry, composition and characterization of the ligands

The sulfonamide derived ligands (**L_1**–**L_5**) were prepared as shown in Scheme 1. All ligands were only soluble in DMF, DMSO and dioxane. The composition of the ligands was consistent with the microanalytical data. The ^{1}H NMR spectral data along with assignments are given in the Experimental, and reveal the appearance[15] of the azomethine proton (–CH═N) signal at 7.69–7.76 ppm. This is further supported[16] by the appearance of a band for ν (C═N) (azomethine) at 1592–1597 cm^{-1} in the IR spectrum of the ligands.



Scheme 1. Preparation of ligands.



Scheme 2. Structure of the metal (II) complexes.

Chemistry, composition and characterization of the metal complexes

The metal (II) complexes (**1**–**20**) of the ligands (**L_1**–**L_5**) were prepared according to the following equation:

$$M(Cl_2) + 2L \xrightarrow[L = L_1 - L_5]{} [M(L)_2Cl_2]$$

Some physical properties such as melting points and percentage yields are given in Table 1.

Conductance and magnetic susceptibility measurements

The molar conductance values (in DMF) fell within the range 11–18 Ω^{-1} cm^2 mol^{-1} for all complexes, showing their non-electrolytic[17] nature. This in turn suggested that the chloride ions are coordinated with the metal ions. The room temperature magnetic moment values of the complexes are given in Table 1. The observed magnetic moment (4.90–4.93 B.M.) is consistent with half-spin octahedral cobalt(II) complexes. The magnetic moment values (1.72–1.92 B.M.) measured for the copper (II) complexes lie in the range expected for a d^9-system, which contains one unpaired electron with octahedral geometry.[18] The measured values (3.18–3.26 B.M.) for the nickel(II) complexes suggest[19] octahedral geometry for these complexes. The zinc(II) complexes were found to be diamagnetic[20] as expected.

IR spectra

The important IR spectral bands of the ligands and its metal complexes are given in the Experimental and in Table 1. Potential electron pair donor sites of synthesized ligands are the pyrrolyl nitrogen/thienyl sulfur, the azomethine nitrogen, the sulfonamide oxygens, the sulfonamide nitrogen, the pyrimidine nitrogens and isoxazole nitrogen/oxygen.

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

No		m.p. (°C)	Yield (%)	B.M. (μ_{eff})	IR (cm^{-1})	λ_{max} (cm^{-1})	Calcd (found) % C	H	N
1.	$[Co(L_1)Cl_2]$ [840.67] $C_{34}H_{34}CoCl_2N_{10}O_4S_2$	260–262	71	4.90	3234 (NH), 1567 (C═N), 1337 (C–N), 1330, 1145 (SO_2), 965 (S–N), 848 (C–S), 432 (M–N), 520 (M–N), 318 (M–Cl)	7280, 17,370, 20,465, 29,305	48.58 (48.61)	4.08 (3.50)	16.66 (16.53)
2.	$[Cu(L_1)Cl_2]$ [845.29] $C_{34}H_{34}CuCl_2N_{10}O_4S_2$	249–251	74	1.74	3230 (NH), 1569 (C═N), 1339 (C–N), 1330, 1145 (SO_2), 965 (S–N), 848 (C–S), 434 (M–N), 525 (M–N), 318 (M–Cl)	14,825, 19,245, 30,235	48.31 (48.44)	4.05 (3.97)	16.57 (16.45)
3.	$[Ni(L_1)Cl_2]$ [840.43] $C_{34}H_{34}NiCl_2N_{10}O_4S_2$	265–267	72	3.18	3230 (NH), 1568 (C═N), 1340 (C–N), 1330, 1145 (SO_2), 965 (S–N), 848 (C–S), 432 (M–N), 530 (M–N), 318 (M–Cl)	10,470, 15,715, 26,430, 29,955	48.59 (48.81)	4.08 (3.78)	16.67 (16.56)
4.	$[Zn(L_1)Cl_2]$ [847.13] $C_{34}H_{34}ZnCl_2N_{10}O_4S_2$	270–272	70	Dia	3230 (NH), 1569 (C═N), 1352 (C–N) 1330,, 1145 (SO_2), 965 (S–N), 848 (C–S), 435 (M–N), 528 (M–N), 318 (M–Cl)	28,445	48.21 (48.33)	4.05 (3.92)	16.53 (16.44)
5.	$[Co(L_2)Cl_2]$ [818.62] $C_{32}H_{32}CoCl_2N_8O_6S_2$	266–268	72	4.91	3230 (NH), 1572 (C═N), 1363 (C–N), 1332, 1143 (SO_2), 968 (S–N), 846 (C–S), 440 (M–N), 532 (M–N), 318 (M–Cl)	7460, 17,510, 20,665, 29,375	46.95 (46.80)	3.94 (3.86)	13.69 (13.63)
6.	$[Cu(L_2)Cl_2]$ [823.24] $C_{32}H_{32}CuCl_2N_8O_6S_2$	261–263	71	1.92	3230 (NH), 1571 (C═N), 1360 (C–N), 1332, 1143 (SO_2), 968 (S–N), 846 (C–S), 427 (M–N), 534 (M–N), 318 (M–Cl)	15,150, 19,410, 30,310	46.69 (46.87)	3.92 (3.98)	13.61 (13.56)
7.	$[Ni(L_2)Cl_2]$ [818.38] $C_{32}H_{32}NiCl_2N_8O_6S_2$	240–243	69	3.24	3230 (NH), 1571 (C═N), 1356 (C–N), 1332, 1143 (SO_2), 968 (S–N), 846 (C–S), 435 (M–N), 538 (M–N), 318 (M–Cl)	10,515, 15,860, 26,565, 30,115	46.97 (46.77)	3.94 (3.62)	13.69 (13.58)
8.	$[Zn(L_2)Cl_2]$ [825.08] $C_{32}H_{32}ZnCl_2N_8O_6S_2$	257–259	77	Dia	3230 (NH), 1572 (C═N), 1362 (C–N), (SO_2), 968 (S–N), 1332, 1143 846 (C–S), 430 (M–N), 534 (M–N), 318 (M–Cl)	29,135	46.58 (46.61)	3.91 (3.48)	13.58 (13.69)
9.	$[Co(L_3)Cl_2]$ [818.67] $C_{30}H_{24}CoCl_2N_8O_4S_4$	235–237	74	4.92	3230 (NH), 1573 (C═N), 1343 (C–S), 1329, 1144 (SO_2), 967 (S–N), 850 (C–S), 440 (M–N), 545 (M–S), 318 (M–Cl)	7355, 17,480, 20,450, 29,315	44.01 (44.08)	2.95 (2.89)	13.69 (13.76)
10.	$[Cu(L_3)Cl_2]$ [823.28] $C_{30}H_{24}CuCl_2N_8O_4S_4$	240–242	75	1.84	3230 (NH), 1572 (C═N), 1347 (C–S), 1329, 1144 (SO_2), 967 (S–N), 850 (C–S), 436 (M–N), 540 (M–S), 318 (M–Cl)	14,945, 19,270, 30,275	43.77 (43.70)	2.94 (2.85)	13.61 (13.76)
11.	$[Ni(L_3)Cl_2]$ [818.43] $C_{30}H_{24}NiCl_2N_8O_4S_4$	233–235	71	3.22	3230 (NH), 1574 (C═N), 1345 (C–S), 1329, 1144 (SO_2), 967 (S–N), 850 (C–S), 428 (M–N), 535 (M–S), 318 (M–Cl)	10,520, 15,785, 26,555, 30,110	44.03 (44.16)	2.96 (3.05)	13.69 (13.58)
12.	$[Zn(L_3)Cl_2]$ [825.13] $C_{30}H_{24}ZnCl_2N_8O_4S_4$	247–249	68	Dia	3230 (NH), 1573 (C═N), 1349 (C–S), 1329, 1144 (SO_2), 967 (S–N), 850 (C–S), 440 (M–N), 537 (M–S), 318 (M–Cl)	28,435	43.67 (43.62)	2.93 (2.99)	13.58 (13.55)

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Table 1. (*Continued*)

No		m.p. (°C)	Yield (%)	B.M. (μ_{eff})	IR (cm^{-1})	λ_{max} (cm^{-1})	Calcd (found) % C	H	N
13.	$[Co(L_4)Cl_2]$ [852.73] $C_{32}H_{30}CoCl_2N_6O_6S_4$	243–245	75	4.93	3230 (NH), 1577 (C═N), 1355 (C–S), 1330, 1145 (SO_2), 968 (S–N), 846 (C–S), 442 (M–N), 522 (M–S), 318 (M–Cl)	7475, 17,515, 20,620, 29,380	45.07 (45.28)	3.55 (3.49)	9.86 (10.03)
14.	$[Cu(L_4)Cl_2]$ [857.34] $C_{32}H_{30}CuCl_2N_6O_6S_4$	240–242	73	1.91	3230 (NH), 1574 (C═N), 1360 (C–S), 1330, 1145 (SO_2), 968 (S–N), 846 (C–S), 440 (M–N), 529 (M–S), 318 (M–Cl)	15,115, 19,400, 30,315	44.83 (44.72)	3.53 (3.57)	9.80 (10.07)
15.	$[Ni(L_4)Cl_2]$ [852.48] $C_{32}H_{30}NiCl_2N_6O_6S_4$	251–253	71	3.26	3230 (NH), 1576 (C═N), 1345 (C–S), 1330, 1145 (SO_2), 968 (S–N), 846 (C–S), 438 (M–N), 531 (M–S), 318 (M–Cl)	10,475, 15,850, 26,510, 29,915	45.09 (45.02)	3.55 (3.43)	9.86 (9.78)
16.	$[Zn(L_4)Cl_2]$ [859.18] $C_{32}H_{30}ZnCl_2N_6O_6S_4$	256–258	78	Dia	3230 (NH), 1575 (C═N), 1340 (C–S), 1330, 1145 (SO_2), 968 (S–N), 846 (C–S), 439 (M–N), 535 (M–S), 318 (M–Cl)	28,730	44.73 (44.70)	3.52 (3.55)	9.78 (9.88)
17.	$[Co(L_5)Cl_2]$ [874.78] $C_{34}H_{32}CoCl_2N_8O_4S_4$	248–250	76	4.91	3230 (NH), 1577 (C═N), 1352 (C–S), 1333, 1147 (SO_2), 965 (S–N), 847 (C–S), 435 (M–N), 545 (M–S), 318 (M–Cl)	7390, 17,365, 20,565, 29,295	46.68 (46.76)	3.69 (3.92)	12.81 (12.88)
18.	$[Cu(L_5)Cl_2]$ [879.39] $C_{34}H_{32}CuCl_2N_8O_4S_4$	259–261	79	1.75	3230 (NH), 1572 (C═N), 1355 (C–S), 1333, 1147 (SO_2), 965 (S–N), 847 (C–S), 433 (M–N), 543 (M–S), 318 (M–Cl)	14,820, 19,335, 30,230	46.44 (46.51)	3.67 (3.48)	12.74 (12.81)
19.	$[Ni(L_5)Cl_2]$ [874.53] $C_{34}H_{32}NiCl_2N_8O_4S_4$	255–257	74	3.21	3230 (NH), 1575 (C═N), 1342 (C–S), 1333, 1147 (SO_2), 965 (S–N), 847 (C–S), 435 (M–N), 540 (M–S), 318 (M–Cl)	10,470, 15,710, 26,440, 30,205	46.70 (46.45)	3.69 (3.72)	12.81 (12.77)
20.	$[Zn(L_5)Cl_2]$ [881.23] $C_{34}H_{32}ZnCl_2N_8O_4S_4$	260–262	77	Dia	3230 (NH), 1573 (C═N), 1340 (C–S), 1333, 1147 (SO_2), 965 (S–N), 847 (C–S), 432 (M–N), 538 (M–S), 318 (M–Cl)	29,111	46.34 (46.49)	3.66 (3.58)	12.72 (12.61)

In the IR spectra of the ligands a sharp band observed in the range of 1592–1597 cm^{-1} and a medium sharp band at 1390–1397 cm^{-1} were assigned[21] to the ν (C═N) mode and ν (C–N)/ν (C–S) stretching of pyrrolyl/thienyl 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 to lower frequency by 20–30 cm^{-1} (1567–1577 cm^{-1}) in all of the complexes. This is further confirmed by the appearance of the new bands at 427–442 cm^{-1} due to the ν (M–N) band.[22]

The coordination through the pyrrolyl ring nitrogen/thienyl ring sulfur was revealed by shifting of the C–N/C–S band to much lower frequencies (1337–1363 cm^{-1}) in all the complexes as compared with that of the ligands. This was further confirmed by the appearance of the new band at 520–545 cm^{-1} due to ν (M–N)/ν (M–S) in all the complexes. The bands in the ligand due to $\nu_{asymm}(SO_2)$ and $\nu_{symm}(SO_2)$ appeared at 1329–1333 and 1143–1147 cm^{-1}, respectively.[23] These bands remained almost unchanged in the complexes, indicating that this group does not participate in coordination. This is supported by the unchanged ν (S–N) and ν (C–S) modes appearing at 965–968 and 846–850 cm^{-1}, respectively,[24] in the ligands after complexation. Also, the band due to ν (–N═) pyrimidine or isoxazole ring appearing in the range of 1549–1555 cm^{-1} did not show any appreciable change on complexation, suggesting that these ring nitrogens of these moieties do not take part in coordination. A new band appearing at 318 cm^{-1} assigned[25] 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.

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^{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 were given in the Experimental. All the protons due to heteroaromatic/aromatic groups were found to be in their expected region.[26] The conclusions drawn from these studies lend further support to the mode of bonding discussed with regard to their IR spectra. The coordination of the azomethine nitrogen is inferred by the downfield shifting of the –C*H*═N– proton signal from 7.69–7.76 ppm in the ligands to 8.49–8.58 ppm in the complexes. Also, the pyrrolyl/thienyl protons underwent downfield shift by about 0.5–0.8 ppm due to the increased conjugation[27] and coordination of pyrrolyl ring nitrogen/thienyl ring sulfur to the metal atom. 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 were given in the Experimental. The carbons atoms due to heteroaromatic/aromatic groups were found to be in their expected region. The conclusions drawn from these studies present further support for 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 near coordination sites underwent downfield shifting by 4.0–8.0 ppm due to the increased conjugation and coordination with the metal atoms. Furthermore, the number of carbons agrees well with the expected values.

Electronic spectra

The Co(II) complexes exhibited well-resolved, low-energy bands at 7280–7475 cm^{-1}, 17, 365–17, 515 cm^{-1} and a strong high-energy band at 20, 450–20, 665 cm^{-1} (Table 1) which were assigned[18] to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ for a high-spin octahedral geometry.[19] A high-intensity band at 29, 295–29, 380 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[28] consistency with their octahedral environment.

The electronic spectra of the Cu(II) complexes (Table 1) showed two low-energy weak bands at 14, 820–15, 150 cm^{-1} and 19, 245–19, 410 cm^{-1} and a strong high-energy band at 30, 230–30, 315 cm^{-1} and may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively.[29] 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.[30]

The electronic spectra of the Ni(II) complexes showed d–d bands in the regions 10,470–10,520, 15,710–15,850 and 26, 430–26, 565 cm^{-1}. These were assigned[31] 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, 915–30, 205 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements showed two unpaired electrons per Ni(II) ion, suggesting[29] also an octahedral geometry for the Ni(II) complexes. The electronic spectra of the Zn(II) complexes exhibited only a high-intensity band at 28, 435–29, 135 cm^{-1} and were assigned[30] to a ligand–metal charge transfer.

Biological activity

Antibacterial bioassay

All compounds were tested against four Gram-negative (*E. coli, S. flexenari, P. aeruginosa* and *S. typhi*) and two

Table 2. Results of antibacterial bioassay (concentration used 1 mg ml^{-1} of DMSO)

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

(*a*) = *E.coli*, (*b*) = *S.flexenari*, (*c*) = *P.aeruginosa*, (*d*) = *S.typhi*, (*e*) = *S.aureus*, (*f*) = *B.subtilis*.
<10: weak; 10–16: moderate; >16: significant. SD = standard drug (imipenum).

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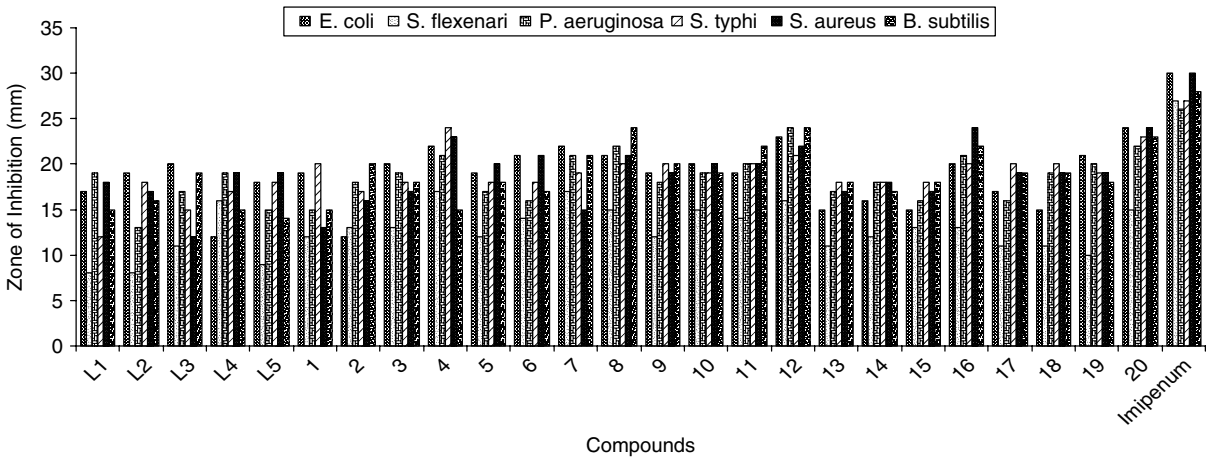

Figure 1. Comparison of antibacterial activity.

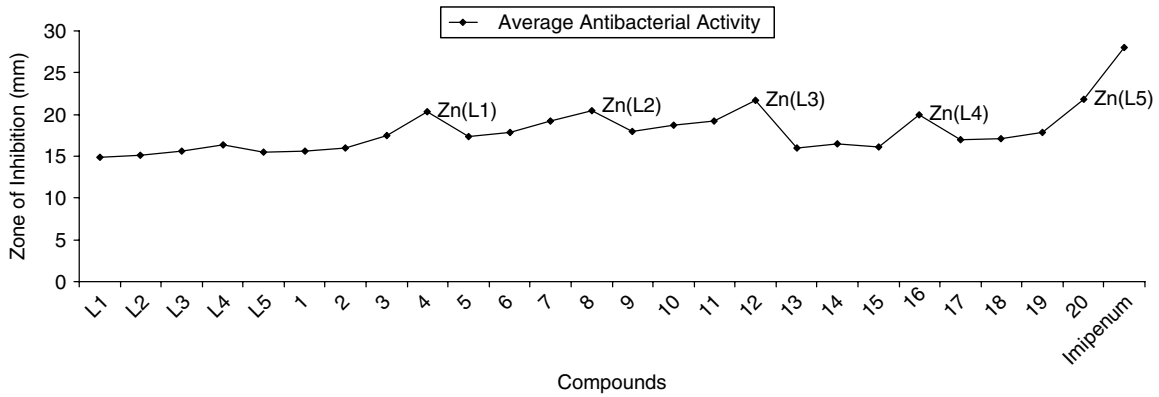

Figure 2. Average antibacterial activity of ligands vs metal (II) complexes.

Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains (Table 2) according to the literature protocol.[10,11] The results were compared with those of the standard drug imipenum (Fig. 1). All ligands showed moderate to significant activity against all Gram-negative and Gram-positive bacterial strains except against *S. flexenari* (*b*), which showed a week activity. Compounds **1–20** exhibited overall a significant activity against *E. coli, P. aeruginosa, S. typhi, B. subtilis* and *S. aureus*. However a moderate activity was observed of compound **1** against *S. flexenari, P. aeruginosa* and *S. aureus*, **2** against *E. coli* and *S. flexenari*, **4** against *B. subtilis*, **7** against *S. aureus*, and **3, 5, 6, 8–11, 13–20** against *S. flexenari.* It was evident that overall potency of uncoordinated compounds was enhanced on coordination with metal ions. However the zinc(II) complexes of all the ligands were observed to be the most active against various species (Fig. 2).

Antifungal bioassay

The antifungal screening of all compounds was carried out against *T. longifusus, C. albican, A. flavus, M. canis, F. solani* and *C. glaberata* fungal strains according to the literature protocol.[12] The inhibition results (mm) were compared with the results of inhibition of standard drugs miconazole and amphotericin B (Fig. 3). These results, illustrated in Table 3, indicate that compound $\mathbf{L_1}$ showed significant activity against (*b*), (*c*) and (*e*), $\mathbf{L_2}$ showed significant activity against (*a*), (*c*), (*d*) and (*e*), $\mathbf{L_3}$ showed significant activity against (*a*) and (*d*), $\mathbf{L_4}$ showed significant activity against (*a*) and (*b*), $\mathbf{L_5}$ showed significant activity against (*b*) and (*e*), **1** showed significant activity against (*a*), (*c*) and (*d*), **2** showed significant activity against (*d*) and (*f*), **3** showed significant activity against (*a*), (*b*), (*c*), (*e*)and (*f*), **4** showed significant activity against (*b*) and (*c*), **5** showed significant activity against (*b*), (*d*) and (*e*), **6** showed significant activity against (*b*) and (*e*), **7** showed significant activity against (*d*) and (*f*), **8** showed significant activity against (*b*), **9** showed significant activity against (*d*), (*e*) and (*f*), **10** showed significant activity against (*a*) and (*f*), **11** showed significant activity against (*a*), (*b*) and (*f*), **12** showed significant activity against (*a*) and (*f*), **13** showed significant activity against (*c*), (*d*) and (*e*), **14** showed significant activity against (*c*), (*d*) and (*f*), **15** showed significant activity against (*a*) and (*d*), **16** showed significant activity against (*c*) and (*f*), **17** showed significant

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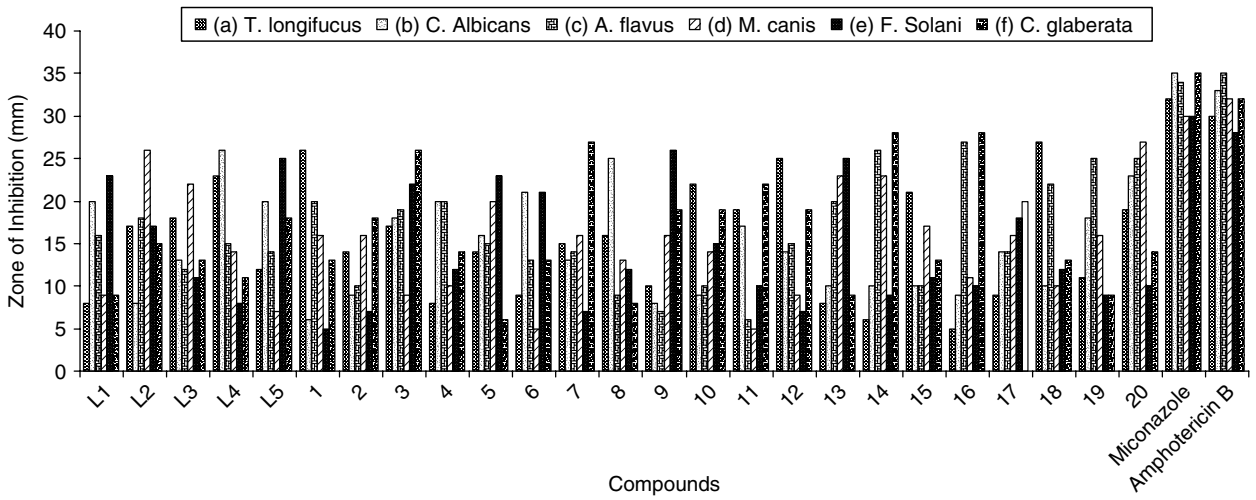

Figure 3. Comparison of antifungal activity.

Table 3. Results of antifungal bioassay (concentration used 200 μg ml^{-1})

	Compound (zone of inhibition in mm)																									
Organism	L_1	L_2	L_3	L_4	L_5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SD-1	SD-2
(a)	08	17	18	23	12	26	14	17	08	14	09	15	16	10	22	19	25	08	06	21	05	09	27	11	19	32	30
(b)	20	08	13	26	20	06	09	18	20	16	21	13	25	08	09	17	14	10	10	10	09	14	10	18	23	35	33
(c)	16	18	12	15	14	20	10	19	20	15	13	14	09	07	10	06	15	20	26	10	27	14	22	25	25	34	35
(d)	09	26	22	14	07	16	16	09	10	20	05	16	13	16	14	05	09	23	23	17	11	16	10	16	27	30	32
(e)	23	17	11	08	25	05	07	22	12	23	21	07	12	26	15	10	07	25	09	11	10	18	12	09	10	30	28
(f)	09	15	13	11	18	13	18	26	14	06	13	27	08	19	19	22	19	09	28	13	28	20	13	09	14	35	32

(*a*) = *T.longifucus*, (*b*) = *C.Albicans*, (*c*) = *A.flavus*, (*d*) = *M.canis*, (*e*) = *F.Solani*, (*f*) = *C.glaberata*.
<10: weak; 10–16: moderate; >16: significant. SD-1 = standard drug (miconazole); SD-2 = standard drug (amphotericin B).

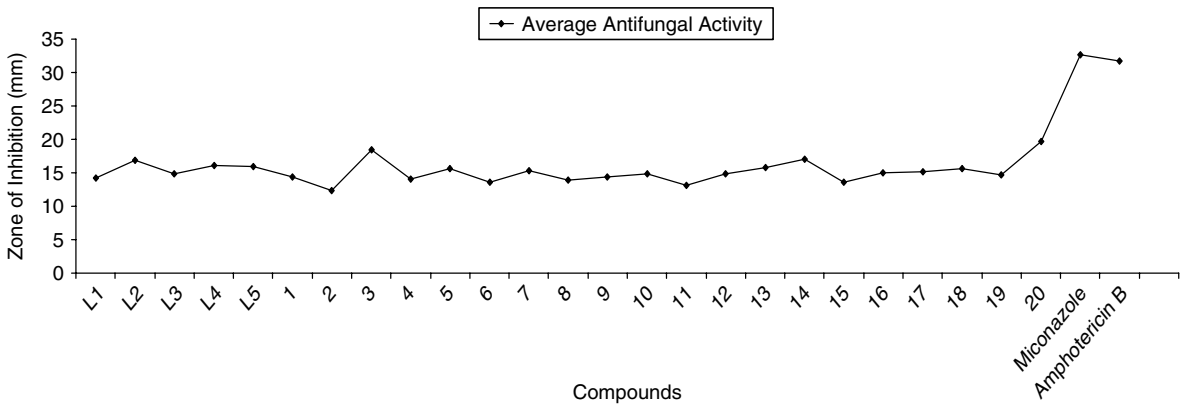

Figure 4. Average antifungal activity in ligands vs metal (II) complexes.

activity against (*d*), (*e*) and (*f*), **18** showed significant activity against (*a*) and (*c*), **19** showed significant activity against (*b*), (*c*) and (*d*), and **20** showed significant activity against (*b*), (*c*) and (*d*) fungal strains. The effect of metal complexation on antifungal activity of the ligands can be seen (Fig. 4).

Minimum inhibitory concentration for antibacterial activity

The preliminary antibacterial screening showed that compounds **4, 8, 12, 16** and **20** were the most active ones (above 80%). These compounds were therefore selected for antibacterial MIC studies (Table 4).

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Table 4. Results of minimum inhibitory concentration (M ml^{-1}) of the selected compounds (**4, 8, 12, 16** and 20) against selected bacteria

	4	8	12	16	20
Gram-negative					
E.coli	—	—	—	—	1.1347×10^{-8}
P. aeruginosa	5.9023×10^{-8}	1.2120×10^{-8}	1.2119×10^{-7}	5.819×10^{-8}	5.6738×10^{-8}
S. typhi	2.9511×10^{-8}	—	—	—	2.8369×10^{-8}
Gram-positive					
S. aureus	—	—	—	2.9097×10^{-8}	1.1347×10^{-7}
B. subtilis	—	6.0600×10^{-8}	6.0596×10^{-8}	—	1.1347×10^{-8}

Table 5. Brine shrimp bioassay data of the ligands (L_1–L_5) and their metal(II) complexes (**1**–**20**)

Compound	LD_{50} (M ml^{-1})
L_1	$>2.8135 \times 10^{-3}$
L_2	$>2.9036 \times 10^{-3}$
L_3	$>2.9034 \times 10^{-3}$
L_4	$>2.7667 \times 10^{-3}$
L_5	$>2.6847 \times 10^{-3}$
1	$>1.1895 \times 10^{-3}$
2	$>1.1830 \times 10^{-3}$
3	$>1.1898 \times 10^{-3}$
4	$>1.1804 \times 10^{-3}$
5	$>1.2215 \times 10^{-3}$
6	$>1.2147 \times 10^{-3}$
7	$>1.2219 \times 10^{-3}$
8	$>1.2120 \times 10^{-3}$
9	$>1.2214 \times 10^{-3}$
10	$>1.2146 \times 10^{-3}$
11	$>1.2218 \times 10^{-3}$
12	$>1.2119 \times 10^{-3}$
13	$>1.1727 \times 10^{-3}$
14	5.5637×10^{-4}
15	$>1.1730 \times 10^{-3}$
16	$>1.1639 \times 10^{-3}$
17	$>1.1431 \times 10^{-3}$
18	$>1.1371 \times 10^{-3}$
19	4.4023×10^{-4}
20	$>1.1347 \times 10^{-3}$

Cytotoxic bioassay

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al*.[13] From the data recorded in Table 5, it is evident that only two compounds, (**14** and **19**) displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive for this assay. Compound **14** showed activity ($LD_{50} = 5.5637 \times 10^{-4}$ M ml^{-1}) in the present series of compounds, whereas the other active compound (**19**) of the series demonstrated activity, $LD_{50} = 4.4023 \times 10^{-4}$ M ml^{-1}.

This enhancement in the activity of L_1—L_5 may be rationalized on the basis of their structures. It has been suggested that chelation/coordination reduces the polarity of the metal ion[32–35] because of the partial sharing of its positive charge with the donor groups and possibly the π-electron delocalization within the whole chelate ring system thus formed during coordination. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn favors[36–38] its permeation through the lipoid layer of the membrane. It has also been observed that some moieties such as azomethine linkage or heteroaromatic system introduced to such compounds exhibit[39] extensive biological activities that may be responsible for the increase of hydrophobic character and liposolubility of the molecules in crossing the cell membrane of the micro-organism and hence enhance the biological utilization ratio and activity of the compounds.

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