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Metal-based isatin-bearing sulfonamides: their synthesis, characterization and biological properties

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A new series of antibacterial and antifungal isatin bearing sulfonamides and their cobalt (II), copper (II), nickel (II) and zinc (II) metal complexes have been synthesized, characterized and screened for their *in vitro* antibacterial activity against *Bacillus cereus, Corynebacterium diphtheriae, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Shigilla dysentriae* and *Staphylococcus aureus* and for *in vitro* antifungal activity against *Trichophyton schoenleinii, Candid glabrata, Pseudallescheria boydii, Candida albicans, Aspergillus niger, Microsporum canis* and *Trichophyton mentagrophytes*. The results of these studies revealed that all compounds showed moderate to significant antibacterial activity. The brine shrimp bioassay was also carried out to study their *in vitro* cytotoxic properties. Only three compounds, 2, 11 and 22 displayed potent cytotoxic activity as $LD_{50} = 1.56 \times 10^{-7}$, 1.59×10^{-7} and 1.67×10^{-7} M/ml respectively, against *Artemia salina*.

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

INTRODUCTION

Isatin (2,3-indolinone) compounds have been long known as valuable synthons in the preparation of biologically active compounds.[1–6] These compounds possess a wide spectrum of medicinal properties and thus have been studied for potential activity against tuberculosis,[7,8] leprosy,[9] fungal,[10–13] viral[14] and bacterial[13,15] infections, rheumatism,[16] trypanocomiasis[17] and convulsions.[18–21] Because of the varied significant biological activities possessed by known sulfonamide classes of antibacterial agent, it was thought worthwhile to combine the chemistry of sulfonamides with that of isatins. As such we have been able to prepare novel isatin-bearing sulfonamides ($\mathbf{L_1}$–$\mathbf{L_6}$) (Fig. 1), which are expected to be of medicinal importance. Keeping in view the promising use of potentially metal-based antibacterial/antifungal/antiviral therapy that has provoked wide interest[5–14] in this diversified area, we report some metal-based [(Co (II), Cu (II), Ni (II) and Zn (II)] compounds (**1–24**) incorporated with newly synthesized isatin-bearing sulfonamides and their *in vitro* antibacterial/antifungal application. The group of these compounds has been fully characterized on the basis of their IR, NMR, UV spectral and elemental analyses. These compounds and their metal complexes have been found to possess a wide spectrum of antibacterial activity against various human pathogenic species, e.g. *B. cereus, C. diphtheriae, E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, S. typhi, S. dysentriae* and *S. aureus* and antifungal activity against human and animal pathogens such as *T. schoenleinii, C. glabrata, P. boydii, C. albicans, A. niger, M. canis* and *T. mentagrophytes*, respectively. The metal complexes were found to show much enhanced activity against two or more bacterial strains on comparison with the uncomplexed simple ligands.

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EXPERIMENTAL

Materials and methods

Solvents used were analytical grades; all metals (II) were used as chloride salts. IR spectra 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 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 and are not corrected. The complexes were analyzed for their metal contents by EDTA titration.[22]

General method for the preparation of ligands (L_1–L_6)

To a stirred solution of the respective sulfonamide (0.005 mol) was added the respective isatin (0.005 mol). The mixture was refluxed. The precipitates formed during reflux were cooled to room temperature and collected by suction filtration. Washing thoroughly with ethanol afforded TLC-pure products in good yield. The reactant solvent, refluxing time, colour of the product and yield of every ligand are individually given in Scheme 1. *N*-methylisatin,[23] *N*-acetylisatin[24] and *N*-propionylisatin[25] were prepared by the reported method.

4-(2-Oxo-1,2-dihydro-indol-3-ylideneamino) benzenesulfonamide (L_1)

Melting point: 270–271 °C. IR (KBr, cm^{-1}): 3320 (NH_2), 3235 (NH), 1715 (C═O), 1585 (C═N), 1325, 1140 (S═O), 960 (S–N), 845 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.28–7.46 (m, 4H, indole), 7.75–7.81 (m, 4H, benzene), 7.88 (s, 2H, SO_2NH_2), 10.27 (s, 1H, NH). Anal. calcd for $C_{14}H_{11}N_3O_3S$ (301.32): C, 55.80; H, 3.68; N, 13.94. Found: C, 56.16; H, 3.32; N, 13.88%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 7.32–7.53 (m, 4H, indole), 7.79–7.97 (m, 4H, benzene), 8.14 (s, 2H, SO_2NH_2), 10.32 (s, 1H, NH).

4-[2-(2-Oxo-1,2-dihydro-indol-3-ylideneamino) ethyl]-benzenesulfonamide (L_2)

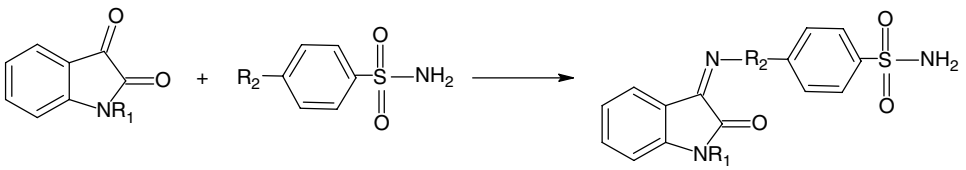
Melting point: 170–180 °C (decompose). IR (KBr, cm^{-1}): 3320 (NH_2), 3235 (NH), 1715 (C═O), 1585 (C═N), 1325, 1140 (S═O), 960 (S–N), 845 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.13 (t, 2H, CH_2–benzene), 3.47 (dd, 2H, –CH_2–N), 7.65–7.72 (m, 4H, indole), 7.75–7.81 (m, 4H, benzene), 7.87 (s, 2H, SO_2NH_2), 10.27 (s, 1H, NH). Anal. calcd for $C_{16}H_{15}N_3O_3S$ (329.37): C, 58.34; H, 4.59; N, 12.76. Found: C, 58.66; H, 4.32; N, 12.58%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 3.18 (t, 4H, CH_2–benzene), 3.52 (dd, 2H, CH_2–N), 7.71–7.78 (m, 4H, indole), 7.80–7.86 (m, 4H, benzene), 7.91 (s, 2H, SO_2NH_2), 10.32 (s, 1H, NH).

4-(1-Methyl-2-oxo-1,2-dihydro-indol-3-ylidene-amino)benzenesulfonamide (L_3)

Melting point: 256–257 °C. IR (KBr, cm^{-1}): 3320 (NH_2), 1715 (C═O), 1585 (C═N), 1325, 1140 (S═O), 960 (S–N), 845 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.86 (s, 3H, N–CH_3), 7.28–7.46 (m, 4H, indole), 7.75–7.81 (m, 4H, benzene), 7.88 (s, 2H, SO_2NH_2). Anal. Calcd. for $C_{15}H_{13}N_3O_3S$ (315.35): C, 57.13; H, 4.16; N, 13.32 Found: C, 57.46; H, 4.58; N, 13.52%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 2.91 (s, 3H, N–CH_3), 7.33–7.52 (m, 4H, indole), 7.79–7.88 (m, 4H, benzene), 7.93 (s, 2H, SO_2NH_2).

4-(1-Acetyl-2-oxo-1,2-dihydro-indol-3-ylidene-amino)benzenesulfonamide (L_4)

Melting point: 235–236 °C. IR (KBr, cm^{-1}): 3320 (NH_2), 1785 ($NCOCH_3$), 1715 (C═O), 1585 (C═N), 1325, 1140 (S═O), 960 (S–N), 845 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.13 (t, 2H, CH_2–benzene), 3.47 (dd, 2H, CH_2–N), 3.21 (s, 3H, $NCOCH_3$), 7.28–7.46 (m, 4H, indole), 7.75–7.81 (m, 2H, benzene), 7.88 (s, 2H, SO_2NH_2). Anal. calcd for $C_{16}H_{13}N_3O_4S$ (343.36): C, 55.97; H, 3.82; N, 12.24. Found: C, 55.76; H, 3.96; N, 12.38%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 3.18 (t, 2H, CH_2–benzene), 3.53 (dd, 2H, CH_2–N), 3.27 (s, 3H, $NCOCH_3$),



No.	R_1	R_2	Solvent	Reflux (h)	Colour	% Yield
L_1	H	N	1-Butanol	10	Orange	77
L_2	H	CH_2CH_2	Ethanol	3	Yellow	67
L_3	CH_3	N	1-Butanol	15	Orange	65
L_4	OCH_3	CH_2CH_2	1, 4-Dioxane	10	Yellow	45
L_5	$COCH_3$	N	1, 4-Dioxane	12	Yellow	85
L_6	$COCH_2CH_3$	N	1, 4-Dioxane	10	Yellow	42

Scheme 1. Formation of the Ligands.

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7.32–7.53 (m, 4H, indole), 7.79–7.87 (m, 2H, benzene), 7.91 (s, 2H, SO_2NH_2).

4-[2-(1-Acetyl-2-oxo-1,2-dihydro-indol-3-ylideneamino)-ethyl] benzenesulfonamide (L_5)

Melting point: 157–158 °C. 3320 (NH_2), 1785 ($NCOCH_3$), 1715 (C═O), 1585 (C═N), 1325, 1140 (S═O), 960 (S–N), 845 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.21 (s, 3H, $NCOCH_3$), 7.28–7.46 (m, 4H, indole), 7.75–7.81 (m, 4H, benzene), 7.88 (s, 2H, SO_2NH_2. Anal. calcd for $C_{18}H_{17}N_3O_4S$ (371.41): C, 58.21; H, 4.61; N, 11.31. Found: C, 58.05; H, 4.32; N, 11.78%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 3.28 (s, 3H, $NCOCH_3$), 7.32–7.53 (m, 4H, indole), 7.81–7.88 (m, 4H, benzene), 7.93 (s, 2H, SO_2NH_2).

4-(2-Oxo-1-propionyl-1,2-dihydro-indol-3-ylideneamino)benzenesulfonamide (L_6)

Melting point: 201–202 °C. 3320 (NH_2), 1780 ($NCOCH_2CH_3$), 1715 (C═O), 1585 (C═N), 1325, 1140 (S═O), 960 (S–N), 845 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.21 (s, 3H, $NCOCH_3$), 3.36 (dd, 2H, $COCH_2$), 7.28–7.46 (m, 4H, indole), 7.75–7.81 (m, 4H, benzene), 7.88 (s, 2H, SO_2NH_2). Anal. calcd for $C_{17}H_{15}N_3O_4S$ (357.38): C, 57.13; H, 4.23; N, 11.76. Found: C, 57.36; H, 4.38; N, 11.32%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 3.28 (s, 3H, $NCOCH_3$), 3.40 (dd, 2H, $COCH_2$), 7.32–7.52 (m, 4H, indole), 7.79–7.87 (m, 4H, benzene), 7.92 (s, 2H, SO_2NH_2).

General method for the preparation of metal (II) complexes (1–24)

To a hot magnetically stirred dioxane (20 ml) solution of the respective sulfonamide (0.02 mol), an aqueous solution of the corresponding metal (II) salt (0.01 M) 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. This solution was filtered, washed with dioxane (2 × 15 ml) then with ethanol and lastly with ether and, dried. Recrystallization from 50% ethanol–dioxane gave the desired products. Unfortunately only microcrystalline powders could be obtained, which were impossible to be used for X-ray structural determinations.

Biological activity

Antibacterial bioassay (in vitro)

All the synthesized ligands (**L_1–L_6**) and their corresponding metal (II) complexes (**1–24**) were screened *in vitro* for their antibacterial activity against *B. cereus, C. diphtheriae, E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, S. typhi, S. dysentriae* and *S. aureus* bacterial strains using agar well diffusion method.[26] Two to 8 h old bacterial inoculums containing approximately 10^4–10^6 colony forming units (CFU)/ml were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centers of at least 24 mm. The recommended concentration (100 µl) 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 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared[27] with the standard drug. 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)

Antifungal activities of all compounds were studied against six fungal cultures, *T. schoenleinii, C. glabrata, P. boydii, C. albicans, A. niger, M. canis* and *T. mentagrophytes* 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 (10 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 as zones of inhibition in mm 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 MIC was determined using the disk diffusion technique[28] by preparing disks containing 10, 25, 50 and 100 M/ml 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[29] 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 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 DMF. From this stock solutions 500, 50 and 5 µg/ml were transferred to nine vials (three for each dilution 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 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 was counted. Data were analyzed by Finney computer program to determine the LD_{50} values.[30]

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RESULTS AND DISCUSSION

The sulfonamide derived ligands ($\mathbf{L_1}$–$\mathbf{L_6}$) were prepared as shown in Scheme 1. All ligands were only soluble in DMF, DMSO and dioxane. The composition of the ligands is consistent with the microanalytical data. This is further supported[31] by the appearance of a band for ν(C═N) (azomethine) at 1585 cm^{-1} in the IR spectrum of the ligands.

Chemistry, composition and characterization of the metal complexes

The metal (II) complexes (**1**–**24**) of the ligands ($\mathbf{L_1}$–$\mathbf{L_6}$) were prepared according to the following equation:

$$MCl_2 + 2\ \text{Ligand}(\mathbf{L_1}\text{–}\mathbf{L_6}) \longrightarrow [M(L)_2Cl_2] \quad \text{or} \quad [M(L_2)]Cl_2$$

$$L = (\mathbf{L_1}\text{–}\mathbf{L_6}) \quad M = \text{Co(II), Ni(II) and Zn(II)} \quad M = \text{Cu(II)}$$

Some physical properties are given in Table 1.

Conductance and magnetic susceptibility measurements

The molar conductance values (in DMF) for cobalt, nickel and zinc complexes fall within the range 10–17 Ω^{-1} cm^2 mol^{-1}, showing their non-electrolytic[32] nature. This, in turn, suggests that the chloride ions are coordinated with the metal ions. However, molar conductance values for copper complexes fall in the range 85–88 Ω^{-1} cm^2 mol^{-1}, suggesting their electrolytic behavior.[33] The room temperature magnetic moment values of the complexes are given in Table 1. The observed magnetic moment (4.89–4.92 B.M.) is consistent with half-spin octahedral cobalt (II) complexes. The magnetic moment values (1.35–1.55 B.M.) measured for the copper (II) complexes lie in the range expected to contain one unpaired electron for square-planar geometry.[34] The measured values (3.18–3.32 B.M.) for the nickel (II) complexes suggest[35] octahedral geometry for these complexes. The zinc (II) complexes were found to be diamagnetic,[36] 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. All ligands contain four potential donor sites: the isatin oxygen, the azomethine nitrogen, the sulfonamide oxygens, the sulfonamide nitrogen and/or, in case of ligands L_1 and L_4, the additional pyrimidine nitrogens and isatin nitrogen. In the IR spectra of the ligands sharp bands observed at 1585 and 1715 cm^{-1} are assigned[37] to the ν(C═N) and ν(C═O) modes. There is evidence of the nitrogen and oxygen bonding of the azomethine (C═N) and carbonyl (C═O) groups to the central metal atom stem from the shift of the ν(C═N) and ν(C═O) frequencies to the lower frequency side by 15–25 cm^{-1} (1570–1585 cm^{-1}) and (1690–1700 cm^{-1}) in all of the metal complexes. This is further confirmed by the appearance of the new bands at 425–440 and 510–545 cm^{-1} due to the ν(M–N) and ν(M–O) bands in all the complexes.[38]

The bands in the ligand due to $\nu_{asymm}(SO_2)$ and $\nu_{symm}(SO_2)$ appear at 1325 and 1140 cm^{-1}, respectively.[39] These bands remain almost unchanged in 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 at 960 and 845 cm^{-1}, respectively,[40] in the ligands after complexation. Also, in ligands the band due to isatin-N ring, $COCH_3$ or $COCH_2CH_3$ and free amino group appearing at 1545, 1785 and 3320 cm^{-1} do not show any appreciable change on complexation, suggesting that the ring nitrogen of isatin and free amino groups are not taking part in coordination. A new band appearing at 315 cm^{-1} assigned[41] to the ν(M–Cl) mode in the cobalt (II), nickel (II) and zinc (II) metal complexes was, however, indicative of the fact that chloride atoms are coordinated with the central metal atom. This band was, however, absent in the copper (II) complexes, suggesting that the chloride atoms are not coordinated with the copper metal ions but stay outside the coordination sphere of the complexes.

^{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 in their expected region.[42] The conclusions drawn from these studies lend further support to the mode of bonding shown in their IR spectra. Also, the isatin protons underwent downfield shifting by 0.5–0.7 ppm due to the increased conjugation[43] and coordination of the isatin moiety with 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.

Electronic spectra

The Co(II) complexes exhibited well-resolved, low-energy bands at 7180–7375 cm^{-1}, 17 265–17 410 cm^{-1} and a strong high-energy band at 20 325–20 570 cm^{-1} (Table 1), which are assigned[36] 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.[44] A high-intensity band at 29 180–29 270 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[45] consistency with their octahedral environment [Fig. 1(A)].

The electronic spectra of the Cu (II) complexes (Table 1) showed two low-energy weak bands at 14 655–15 500 cm^{-1} and 19 155–19 315 cm^{-1} and a strong high-energy band at 30 130–30 255 cm^{-1} and may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively.[46] 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 their square-planar geometry[47] [Fig. 1(B)].

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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	$[Co(L_1)Cl_2]$ [732.48] $C_{28}H_{22}CoN_6O_6S_2Cl_2$	286–287	72	4.89	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	7180, 17 265, 20 325, 29 180	45.91 (45.63)	3.03 (3.40)	11.47 (11.13)
2	$[Cu(L_1)]Cl_2$ [737.10] $C_{28}H_{22}CuN_6O_6S_2Cl_2$	290–292	75	1.35	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O)	14 655, 19 155, 30 130	45.62 (45.84)	3.01 (3.37)	11.40 (11.55)
3	$[Ni(L_1)Cl_2]$ [732.24] $C_{28}H_{22}NiN_6O_6S_2Cl_2$	298–300	77	3.18	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	10 360, 15 610 26 315, 29 925	45.93 (45.81)	3.03 (3.28)	11.48 (11.16)
4	$[Zn(L_1)Cl_2]$ [738.94] $C_{28}H_{22}ZnN_6O_6S_2Cl_2$	294–296	75	Dia	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	28 555	45.51 (45.33)	3.00 (3.12)	11.37 (11.11)
5	$[Co(L_2)Cl_2]$ [788.59] $C_{32}H_{30}CoCl_2N_6O_6S_2$	212–214	76	4.92	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	7375, 17 410, 20 570, 29 270	48.74 (48.58)	3.83 (3.56)	10.66 (10.43)
6	$[Cu(L_2)]Cl_2$ [793.20] $C_{32}H_{30}CuCl_2N_6O_6S_2$	208–210	77	1.55	3230 (NH), 1565 (C═N), 1550 (–N═ring), 13 340 (C–O), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S), 425 (M–N), 535 (M–O)	15 500, 19 315, 30 255	48.46 (48.87)	3.81 (3.48)	10.59 (10.36)
7	$[Ni(L_2)Cl_2]$ [788.35] $C_{32}H_{30}NiCl_2N_6O_6S_2$	215–217	75	3.32	3230 (NH), 1572 (C═N), 1550 (–N═ring), 1345 (C–O), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S), 435 (M–N), 530 (M–O), 315 (M–Cl)	10 555, 15 865, 26 570, 30 235	48.75 (48.96)	3.84 (3.62)	10.66 (10.58)
8	$[Zn(L_2)Cl_2]$ [795.05] $C_{32}H_{30}ZnCl_2N_6O_6S_2$	218–220	73	Dia	3230 (NH), 1570 (C═N), 1550 (–N═ring), 1335 (C–O), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S), 430 (M–N), 530 (M–O), 315 (M–Cl)	29 145	48.34 (48.61)	3.80 (3.78)	10.57 (10.69)

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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
9	$[Co(L_3)Cl_2]$ [760.54] $C_{30}H_{26}CoCl_2N_6O_6S_2$	192–194	75	4.90	3230 (NH), 1565 (C═N), 1550 (–N═ring), 1330 (C–O), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S), 440 (M–N), 510 (M–O), 315 (M–Cl)	7255, 17 380, 20 455, 29 215	47.38 (47.17)	3.44 (3.37)	11.05 (11.32)
10	$[Cu(L_3)]Cl_2$ [765.15] $C_{30}H_{26}CuCl_2N_6O_6S_2$	272–274	76	1.38	3230 (NH), 1572 (C═N), 1550 (–N═ring), 1330 (C–O), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S), 430 (M–N), 525 (M–O)	14 845, 19 270, 30 215	47.09 (47.33)	3.42 (3.26)	10.98 (10.76)
11	$[Ni(L_3)Cl_2]$ [760.30] $C_{30}H_{26}NiCl_2N_6O_6S_2$	279–281	77	3.25	3230 (NH), 1569 (C═N), 1550 (–N═ring), 1360 (C–O), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S), 425 (M–N), 520 (M–O), 315 (M–Cl)	10 440, 15 780, 26 455, 29 975	47.39 (47.41)	3.45 (3.26)	11.05 (11.44)
12	$[Zn(L_3)Cl_2]$ [766.99] $C_{30}H_{26}ZnCl_2N_6O_6S_2$	287–289	75	Dia	3230 (NH), 1565 (C═N), 1550 (–N═ring), 1350 (C–O), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S), 430 (M–N), 515 (M–O), 315 (M–Cl)	28 735	46.98 (46.73)	3.42 (3.31)	10.96 (10.74)
13	$[Co(L_4)Cl_2]$ [816.56] $C_{32}H_{26}CoCl_2N_6O_8S_2$	214–216	76	4.91	3230 (NH), 1570 (C═N), 1550 (–N═ring), 1355 (C–O), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S), 440 (M–N), 530 (M–O), 315 (M–Cl)	7345, 17 405, 20 365, 29 210	47.07 (47.28)	3.21 (3.19)	10.29 (10.53)
14	$[Cu(L_4)]Cl_2$ [821.17] $C_{32}H_{26}CuCl_2N_6O_8S_2$	210–212	75	1.42	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O)	15 415, 19 210, 30 215	46.81 (46.98)	3.19 (3.57)	10.23 (10.37)
15	$[Ni(L_4)Cl_2]$ [816.32] $C_{32}H_{26}NiCl_2N_6O_8S_2$	219–221	77	3.28	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	10 515, 15 780, 26 515, 30 110	47.08 (47.42)	3.21 (3.43)	10.29 (10.43)
16	$[Zn(L_4)Cl_2]$ [823.01] $C_{32}H_{26}ZnCl_2N_6O_8S_2$	227–229	77	Dia	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	28 830	46.70 (46.94)	3.18 (3.55)	10.21 (10.37)
17	$[Co(L_5)Cl_2]$ [872.66] $C_{36}H_{34}CoCl_2N_6O_8S_2$	246–248	77	4.90	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	7350, 17 330, 20 465, 29 210	49.55 (49.26)	3.93 (3.62)	9.63 (9.87)

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18	$[Cu(L_5)]Cl_2$ [877.28] $C_{36}H_{34}CuCl_2N_6O_8S_2$	252–254	73	1.50	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O)	15 470, 19 235, 30 240	49.29 (49.51)	3.91 (3.68)	9.58 (9.63)
19	$[Ni(L_5)Cl_2]$ [872.42] $C_{36}H_{34}NiCl_2N_6O_8S_2$	255–257	72	3.30	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	10 470, 15 845, 26 440, 30 200	49.56 (49.45)	3.93 (3.72)	9.63 (9.77)
20	$[Zn(L_5)Cl_2]$ [879.12] $C_{36}H_{34}ZnCl_2N_6O_8S_2$	250–252	75	Dia	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	29 110	49.18 (43.49)	3.90 (3.58)	9.56 (8.61)
21	$[Co(L_6)Cl_2]$ [844.61] $C_{34}H_{30}CoCl_2N_6O_8S_2$	221–223	74	4.89	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	7335, 17 345, 20 515, 29 195	48.35 (48.58)	3.58 (3.73)	9.95 (9.61)
22	$[Cu(L_6)]Cl_2$ [849.22] $C_{34}H_{30}CuCl_2N_6O_8S_2$	218–220	75	1.52	33 320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O)	14 795, 19 210, 30 215	48.09 (48.34)	3.56 (3.39)	9.90 (9.71)
23	$[Ni(L_6)Cl_2]$ [844.37] $C_{34}H_{30}NiCl_2N_6O_8S_2$	225–227	77	3.23	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	10 470, 15 710, 26 585, 30 110	48.36 (48.16)	3.58 (3.78)	9.95 (9.77)
24	$[Zn(L_6)Cl_2]$ [851.07] $C_{34}H_{30}ZnCl_2N_6O_8S_2$	231–233	72	Dia	3320 (NH_2), 1785 ($COCH_3$), 1570 (C═N), 1690 (C═O), 1545 (–N═ring), 1325, 1140 (SO_2), 960 (S–N), 845 (C–S) 425 (M–N), 525 (M–O), 315 (M–Cl)	28 955	47.98 (47.63)	3.55 (3.18)	9.87 (9.96)

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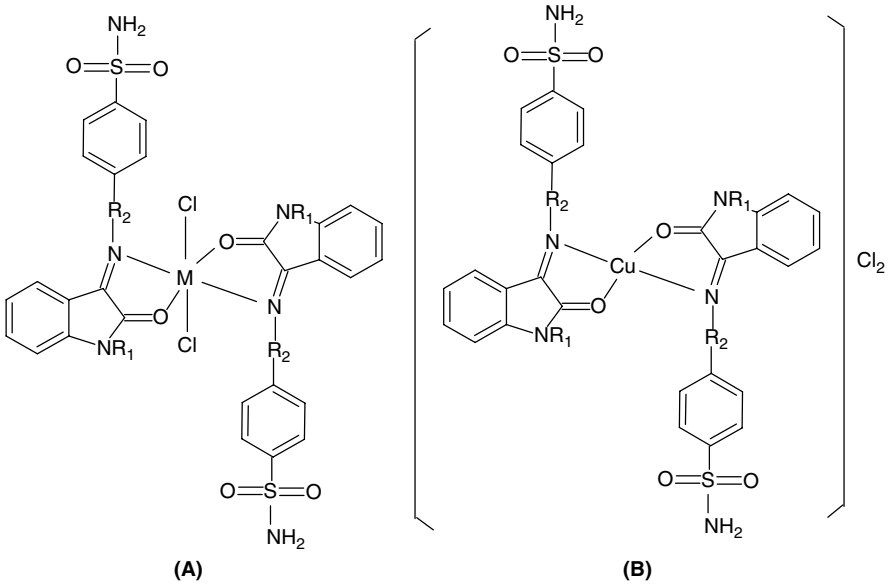

Figure 1. Proposed structure of the metal (II) complexes. M = Co(II), Ni(II) and Zn(II).

The electronic spectra of the Ni (II) complexes showed d–d bands in the region 10 360–10 555, 15 610–15 865 and 26 315–26 570 cm^{-1}. These are assigned[48] 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 29 925–30 235 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements showed two unpaired electrons per Ni (II) ion, also suggesting[46] an octahedral geometry for the Ni (II) complexes [Fig. 1(A)]. The electronic spectra of the Zn (II) complexes exhibited only a high-intensity band at 28 555–29 145 cm^{-1} and are assigned[47] to a ligand–metal charge transfer.

Biological activity

Antibacterial bioassay

All compounds were tested against *B. cereus, C. diphtheriae, E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, S. typhi, S. dysentriae* and *S. aureus* bacterial strains (Table 2) according to literature protocol.[26,27] The results were compared with those of the standard drug imipenum. All ligands showed moderate to significant activity against all bacterial strains except *C. diphtheriae* (**b**) and *S. typhi* (**g**) that showed either a weak or insignificant activity. Compound (**16**) exhibited a significant activity against *B. cereus* (**a**) and overall a moderate activity was observed by all the rest of the compounds against **a**. A significant activity was also observed by compounds $\mathbf{L^4}$, **6, 7, 8** and **13–16** against **a**. All ligands as well as the metal complexes **6–24** showed weak activity against *C. diphtheriae* (**b**) and *S. typhi* (**g**). However a moderate activity was observed by all compounds against bacterial strains: **c, d, e, f, h** and **j**. The zinc (II) complexes of all the ligands were comparatively observed to be the most active against all species. It was interesting to note that methyl and ethyl carbon chain in the ligands and their respective metal chelates had an impact on the bactericidal activity. As the carbon chain of the sulfonamide moiety increased from methyl to ethyl in the ligands $\mathbf{L_2}$ and $\mathbf{L_4}$ and their respective metal complexes **5–8** and **13–16**, the bactericidal activity was also increased as compared to the other ligands and their respective metal complexes.

Antifungal bioassay

The antifungal screening of all compounds was carried out against *T. schoenleinii, C. glabrata, P. boydii, C. albicans, A. niger, M. canis* and *T. mentagrophytes* fungal strains according to the literature protocol.[26] The results were compared with the standard drugs miconazole and amphotericin B. These results illustrated in Table 3 indicate that compounds **12, 22** and **24** showed significant activity against **a, 16** and **22** against **c, 14** and **24** against **d, 9** against **e, 14** against **f** and **16** and **21** against **g** fungal strains.

Minimum inhibitory concentration

The preliminary screening showed that compounds $\mathbf{L_4}$, **5, 6, 7, 8, 13, 14, 15, 16** and **24** were the most active ones above 80%. These compounds were therefore, selected for minimum inhibitory concentration MIC studies (Table 4).

Cytotoxic bioassay

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al*.[29] From the data recorded in Table 5, it is evident that only three compounds, **2, 11** and **22**, displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive for this assay. The compound **22** showed maximum activity ($LD_{50} = 1.56 \times 10^{-7}$ M/ml) in the present series of compounds, whereas

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Table 2. Results of antibacterial bioassay (concentration used 1 mg/ml of DMSO)

	Compound (zone of inhibition in mm)																													
Bacteria	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	17	22	18	24	15	17	19	20	19	20	23	24	25	26	18	19	18	20	25	26	27	27	18	19	19	20	18	19	20	22	30
b	06	08	06	08	06	07	10	09	10	10	12	11	12	14	10	11	12	11	12	13	12	14	10	09	10	12	11	10	11	12	28
c	18	20	19	22	17	18	19	19	19	20	22	20	23	24	20	19	21	20	24	23	20	23	18	19	20	20	19	20	19	20	27
d	16	19	17	19	18	17	18	19	18	18	20	22	22	23	18	19	19	19	20	22	21	23	19	20	18	19	18	19	20	20	29
e	14	17	13	16	13	14	16	18	17	18	19	20	21	22	18	19	18	18	20	19	20	22	18	19	19	18	20	20	21	22	30
f	18	19	18	20	17	17	19	19	20	19	20	20	21	24	18	19	20	19	22	24	26	24	19	18	19	19	20	21	22	23	28
g	05	08	06	09	06	05	07	08	06	07	10	11	09	12	07	08	07	07	10	09	11	11	09	08	07	09	08	07	07	09	29
h	17	23	18	22	18	19	18	19	18	19	24	26	24	25	19	20	18	18	24	24	25	23	19	19	20	19	20	19	21	22	30
j	17	22	18	24	18	19	18	19	18	19	24	23	26	24	18	19	20	20	26	25	26	28	19	20	19	19	20	21	21	22	29

a = *B. cereus*, **b** = *C. diphtheriae*, **c** = *E. coli*, **d** = *K. pneumoniae*, **e** = *P. mirabilis*, **f** = *P. aeruginosa*, **g** = *S. typhi*, **h** = *S. dysentriae*, **j** = *S. aureus*. 10 <: weak; 10–16: moderate; >16: significant. SD = standard drug (imipenum).

Table 3. Results of antifungal bioassay (concentration used 200 μg/ml)

	Compound (% inhibition)																													
Organism	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	00	00	00	00	00	30	00	00	00	00	00	00	00	10	00	28	00	28	00	00	00	05	00	00	00	00	00	30	00	34	A
b	00	07	00	00	00	00	00	00	00	00	00	30	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	32	B
c	15	00	00	00	00	00	00	00	00	25	00	09	00	00	00	10	00	00	00	00	00	32	00	00	00	00	00	32	00	00	C
d	10	00	00	00	00	00	00	14	00	00	00	00	00	00	00	00	00	00	00	35	00	30	00	00	00	00	00	00	00	37	D
e	00	15	00	00	00	00	00	00	00	00	00	00	00	00	32	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	E
f	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	35	00	00	00	00	00	00	00	00	00	00	F
g	00	00	00	00	00	00	00	30	00	00	00	00	00	00	00	00	00	00	00	00	00	35	00	19	00	00	35	00	00	00	G

a = *T. schoenleinii*, **b** = *C. glabrata*, **c** = *P. boydii*, **d** = *C. albicans*, **e** = *A. niger*, **f** = *M. canis*, **g** = *T. mentagrophytes*. SD = standard drugs MIC, μg/ml; A = miconazole (70 μg/ml); B = miconazole (110.8 μg/ml); C = amphotericin B (20 μg/ml); D = miconazole (98.4 μg/ml); E = miconazole (73.25 μg/ml); F = miconazole (110.8 μg/ml); G = miconazole (85.10 μg/ml).

Table 4. Results of minimum inhibitory concentration (M/ml) of the selected compounds (**L_4, 5–8, 13–16** and **24**) against selected bacteria

	L_4	5	6	7	8	13	14	15	16	24
B. cereus	2.91×10^{-7}	1.27×10^{-7}	1.26×10^{-7}	3.17×10^{-8}	3.14×10^{-8}	3.06×10^{-8}	1.22×10^{-8}	1.22×10^{-8}	1.22×10^{-8}	1.17×10^{-7}
E. coli	1.46×10^{-7}	3.17×10^{-8}	1.26×10^{-7}	3.17×10^{-8}	3.14×10^{-8}	6.12×10^{-8}	3.04×10^{-8}	1.22×10^{-7}	3.04×10^{-8}	1.17×10^{-7}
P. aeruginosa	2.91×10^{-7}	6.34×10^{-8}	1.26×10^{-7}	1.27×10^{-7}	6.29×10^{-8}	1.22×10^{-7}	3.04×10^{-8}	1.22×10^{-8}	1.22×10^{-8}	2.94×10^{-8}
S. dysentriae	2.91×10^{-7}	1.27×10^{-7}	1.26×10^{-8}	1.27×10^{-7}	6.29×10^{-8}	1.22×10^{-7}	1.22×10^{-7}	6.12×10^{-8}	6.08×10^{-8}	1.17×10^{-7}
S. aureus	7.28×10^{-8}	3.17×10^{-8}	1.26×10^{-7}	1.27×10^{-8}	3.14×10^{-8}	3.06×10^{-8}	1.22×10^{-8}	3.06×10^{-8}	1.22×10^{-8}	5.87×10^{-8}

Table 5. Brine shrimp bioassay data of the ligands (L_1)–(L_6) and their metal (II) complexes (**1–24**)

Compound	LD_{50} (M/ml)
L_1	$>3.32 \times 10^{-4}$
L_2	$>3.04 \times 10^{-4}$
L_3	$>3.17 \times 10^{-4}$
L_4	$>2.91 \times 10^{-4}$
L_5	$>2.69 \times 10^{-4}$
L_6	$>2.80 \times 10^{-4}$
1	$>1.36 \times 10^{-4}$
2	1.59×10^{-7}
3	$>1.36 \times 10^{-4}$
4	$>1.35 \times 10^{-4}$
5	$>1.27 \times 10^{-4}$
6	$>1.26 \times 10^{-4}$
7	$>1.27 \times 10^{-4}$
8	$>1.26 \times 10^{-4}$
9	$>1.31 \times 10^{-4}$
10	$>1.31 \times 10^{-4}$
11	1.67×10^{-7}
12	$>1.30 \times 10^{-4}$
13	$>1.22 \times 10^{-4}$
14	$>1.22 \times 10^{-4}$
15	$>1.22 \times 10^{-4}$
16	$>1.22 \times 10^{-4}$
17	$>1.14 \times 10^{-4}$
18	$>1.14 \times 10^{-4}$
19	$>1.15 \times 10^{-4}$
20	$>1.14 \times 10^{-4}$
21	$>1.18 \times 10^{-4}$
22	1.56×10^{-7}
23	$>1.18 \times 10^{-4}$
24	$>1.17 \times 10^{-4}$

the other active compounds (**2** and **11**) of the series demonstrated lesser activity ($LD_{50} = 1.59 \times 10^{-7}$ M/ml and 1.67×10^{-7} M/ml) than compound **2**.

The enhancement in antibacterial and antifungal activity on coordination with the metal ions is probably due to the presence of donor systems in the uncoordinated compounds and may inhibit enzyme production, since the enzymes, which require these groups for their activity, appear to be especially more susceptible to deactivation upon coordination/chelation. Chelation reduces the polarity of the metal ion[49–55] 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 favours[56–60] 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 extensive[61–65]

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biological activities that may be responsible for the increased hydrophobic character and liposolubility of the molecules in crossing cell membrane of the micro-organism and hence enhance the biological utilization ratio and activity of the compounds.

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