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In-vitro antibacterial, antifungal and cytotoxic properties of metal-based furanyl derived sulfonamides

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Abstract

A new series of antibacterial and antifungal furanyl-derived 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 four Gram-negative (*Escherichia coli, Shigella flexneri, 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 (II) complexes were found to be comparatively much more active as compared to the others. For antifungal activity generally, compounds (**22**) and (**24**) showed significant activity against *Escherichia coli* (*a*), (**6**) against *Shigella flexeneri* (*b*), (**16**) and (**22**) against *Pseudomonas aeruginosa* (*c*), (**14**) and (**16**) against *Salmonella typhi* (*d*), (**9**) against *Staphylococcus aureus* (*e*) and, (**14**) and (**16**) against *Bacillus subtilis* (*f*) fungal strains. The brine shrimp (*Artemia salina*) bioassay was also carried out to study their *in-vitro* cytotoxic properties. Only three compounds, (**6**), (**10**) and (**23**) displayed potent cytotoxic activity with $LD_{50} = 1.8535 \times 10^{-4}$, 1.8173×10^{-4} and 1.9291×10^{-4} respectively.

Keywords: *Sulfonamides, metal complexes, antibacterial, antifungal, cytotoxicity*

Introduction

The importance of sulfonamides was realized [1] when sulfanilamide, a key analogue of sulfonamide, was reported [2] to be the first antibacterial drug. Later on, many sulfanilamide derivatives were synthesized, characterised and tested for antibacterial [3], anti-tumour [4], anti-carbonic anhydrase [5,6], diuretic [7,8], hypoglycaemic [9], anti-thyroid [10] or protease inhibitory activity [11,12]. Sulfanilamide thus became the foundation for the development and expansion of all other types of medicinally important sulfonamides having a varied spectrum of biological action. Further extension to this significant area was made by the formation of the first silver (I) complex of sulfanilamide which furthermore, emphasised [13] the role of metals in enhancing biological activity. Later on, many other metal complexes of sulfanilamide analogues were subsequently synthesized and investigated for biological activity in detail [14]. Due to the growing interest and increased potential value of these compounds, they also attracted our attention and thus, we have commenced a program, to meticulously explore this potential area of research by designing and investigating the role of metals in some novel sulphonamides prepared in our laboratory. In this connection, we have already reported [15–17] the synthesis of sulfonamide-derived salicylaldehyde

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and their various transition metal complexes along with their evaluation as inhibitors of the physiologically relevant CA isozymes: hCA I and hCA II. In continuation of this work we now report in this paper some other new furanyl-derived sulfonamides and their cobalt (II), copper (II), nickel (II) and zinc (II) compounds. We have also studied their *in vitro* antibacterial activity against four Gram-negative (*E. coli, S. flexneri, P. aeruginosa and S. typhi*) and two Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains and, *in-vitro* antifungal activity against *T. longifusus, C. albicans, A. flavus, M. canis, F. solani* and *C. glaberata.* The brine shrimp bioassay was also carried out to study their *in-vitro* cytotoxic properties.

Materials and methods

Solvents used were analytical grades; all metal (II) were used as chloride salts. IR, NMR, UV-Visible spectra, C, H and N analyses, Conductance and Magnetic measurements were carried out on solid compounds using the respective instruments.

General method for the preparation of ligands ($\mathbf{L_1}$)-($\mathbf{L_6}$)

To a stirred solution of the respective sulfonamide (0.005 mole) in methanol (30 ml) was added furfuraldehyde (0.005 mole) in methanol (10 mL). The mixture was refluxed for 2 h. The precipitates formed during refluxing, were cooled to room temperature and collected by suction filtration. Washing thoroughly with methanol, afforded TLC pure products in good yield.

N-(4,6-Dimethylpyrimidine-2-yl)-4-[furan-2-ylmethylene)amino]benzene- sulfonamide ($\mathbf{L_1}$). Yield 85%; m.p. 232–34°C; IR (KBr, cm^{-1}): 3230 (NH), 1590 (azomethine, HC═N), 1550 (—N═pyrimidine ring), 1385 (C—O), 1325, 1140 (S═O), 960 (S—N), 845 (C—S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.86 (s, 6H, CH_3), 6.43–6.87 (m, 3H, furanyl), 7.73 (s, 1H, azomethine), 7.65–7.72 (m, 4H, N-Ph), 8.32–8.50 (m, 1H, pyrimidine), 11.72 (s, 1H, SO_2HN). Anal. Calcd. for $C_{17}H_{16}N_4O_3S$ (356.39): C, 57.30; H, 4.52; N, 15.72. Found: C, 57.65; H, 4.20; N, 15.58%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 3.24 (s, 6H, CH_3), 7.43–7.58 (m, 3H, furanyl), 7.92–7.96 (m, 4H, N-Ph), 8.57 (s, 1H, azomethine), 8.94–9.31 (m, 1H, pyrimidine), 11.92 (s, 1H, SO_2HN).

4-[(Furan-2-ylmethylene)amino]benzenesulfonamide ($\mathbf{L_2}$). Yield 60%; m.p. 198–99°C; IR (KBr, cm^{-1}): 3230 (NH), 1590 (azomethine, HC═N), 1385 (C—O), 1325, 1140 (S═O), 960 (S—N), 845 (C—S); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.43–6.87 (m, 3H, furanyl), 7.73 (s, 1H, azomethine), 7.65–7.72 (m, 4H, N-Ph), 7.87 (s, 2H, SO_2NH_2). Anal. Calcd. for $C_{11}H_{10}N_2O_3S$ (250.27): C, 52.79; H, 4.03; N, 11.19. Found: C, 52.55; H, 4.36; N, 11.58%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 7.55–7.61 (m, 3H, furanyl), 7.91–7.97 (m, 4H, N-Ph), 8.55 (s, 1H, azomethine), 8.79 (s, 2H, SO_2NH_2).

4-{2-[(Furan-2-ylmethylene)amino]ethyl} benzenesulfonamide ($\mathbf{L_3}$). Yield 66%; m.p. 168–69°C; IR (KBr, cm^{-1}): 3320 (NH_2), 1590 (azomethine, HC═N), 1385 (C—O), 1325, 1140 (S═O), 960 (S—N), 845 (C—S); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.13 (t, 2H, —CH_2-aromatic ring), 3.34 (t, 2H, —CH_2—N), 6.44–6.89 (m, 3H, furanyl), 7.73 (s, 1H, azomethine), 7.65–7.72 (m, 4H, N-Ph), 7.96 (s, 2H, SO_2NH_2). Anal. Calcd. for $C_{13}H_{14}N_2O_3S$ (278.32): C, 56.10; H, 5.07; N, 10.06. Found: C, 56.36; H, 5.32; N, 10.38%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 3.65 (t, 2H, —CH_2-aromatic ring—), 3.86 (t, 2H, —CH_2—N), 7.56–7.85 (m, 3H, furanyl), 7.92–8.25 (m, 4H, N-Ph), 8.88 (s, 2H, SO_2NH_2), 8.54 (s, 1H, azomethine).

4-[(Furan-2-ylmethylene)amino]-N-(5-methylisoxazol-3-yl)benzenesulfonamide ($\mathbf{L_4}$). Yield 73%; m.p: 228–230°C. Yield 66%; m.p. 168–69°C; IR (KBr, cm^{-1}): 3230 (NH), 1590 (azomethine, HC═N), 1385 (C—O), 1325, 1140 (S═O), 960 (S—N), 845 (C—S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.12 (s, 3H, CH_3), 6.43–6.85 (m, 3H, furanyl), 6.97 (dd, 1H, isoxazol), 7.73 (s, 1H, azomethine), 7.55–7.72 (m, 4H, N-Ph), 11.72 (s, 2H, SO_2NH). Anal. Calcd. for $C_{15}H_{13}N_3O_4S$ (331.34): C, 54.37; H, 3.95; N, 12.68. Found: C, 54.46; H, 3.62; N, 12.55%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 2.46 (s, 3H, CH_3), 7.44–7.78 (m, 3H, furanyl), 7.26 (m, 1H, isoxazol), 8.12–8.34 (m, 4H, N-Ph), 8.52 (s, 1H, azomethine), 11.97 (s, 2H, SO_2NH).

4-[(5-Methylfuran-2-ylmethylene)amino]benzenesulfonamide ($\mathbf{L_5}$). Yield 86%; m.p: 232–233°C. IR (KBr, cm^{-1}): 3320 (NH_2), 1590 (azomethine, HC═N), 1385 (C—O), 1325, 1140 (S═O), 960 (S—N), 845 (C—S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.11 (s, 3H, CH_3), 6.43–6.87 (m, 3H, furanyl), 7.73 (s, 1H, azomethine), 7.45–7.68 (m, 4H, N-Ph), 7.85 (s, 2H, SO_2NH_2). Anal. Calcd. for $C_{12}H_{12}N_2O_3S$ (264.30): C, 54.53; H, 4.58; N, 10.60. Found: C, 54.82; H, 4.37; N, 10.88%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 2.57 (s, 3H, CH_3), 7.48–7.76 (m, 3H, furanyl), 8.16–8.36 (m, 4H, N-Ph), 8.94 (s, 2H, SO_2NH_2), 8.68 (s, 1H, azomethine).

4-{2-[(5-Methylfuran-2-ylmethylene)amino]ethyl} benzenesulfonamide ($\mathbf{L_6}$). Yield 62%; m.p: 160–162°C.

IR (KBr, cm^{-1}): 3320 (NH), 1590 (azomethine, HC═N), 1385 (C—O), 1325, 1140 (S═O), 960 (S—N), 845 (C—S); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.10 (s, 3H, CH_3), 3.13 (t, 2H, —CH_2-aromatic ring), 3.34 (t, 2H, —CH_2—N), 6.50–6.83 (m, 3H, furanyl), 7.73 (s, 1H, azomethine), 7.65–7.72 (m, 4H, N-Ph), 11.72 (s, 2H, SO_2NH). Anal. Calcd. for $C_{14}H_{16}N_2O_3S$ (292.35): C, 57.52; H, 5.52; N, 9.58. Found: C, 57.66; H, 5.78; N, 9.37%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 2.54 (s, 3H, CH_3), 3.31 (t, 2H, —CH_2-aromatic ring), 3.58 (t, 2H, —CH_2—N), 7.21–7.53 (m, 3H, furanyl), 8.27–8.46 (m, 4H, N-Ph), 8.64 (s, 1H, azomethine), 11.87 (s, 2H, SO_2NH).

*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 moles), an aqueous solution of the corresponding metal (II) salt (0.01 M) was added. The mixture was refluxed for 2 h, filtered and reduced to half of its volume by evaporation of the solvent *in vacuo*. The concentrated solution was left overnight at room temperature, which led to the formation of a solid product which was filtered, washed with dioxane (2 × 15 mL) then with ether and dried. Recrystallization from 50% aqueous dioxane gave the desired products. Unfortunately only microcrystalline powders could be obtained, which could not 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 four Gram-negative (*E. coli, S. flexneri, P. aeruginosa and S. typhi*) and two Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains using the agar well diffusion method [18]. Two to eight hours 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 centres at least 24 mm. 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 [19] with the standard drug. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with 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, England) was seeded with 10^5 (cfu) mL^{-1} fungal spore suspensions and transferred to petri plates. Discs 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 seven days. The results were recorded as zones of inhibition (in mm) and compared with the standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration (MIC). 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 [20] by preparing discs containing 10, 25, 50 and 100 μg/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 [21] 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 smaller 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 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 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 by a Finney computer program to determine the LD_{50} values [22].

Results and discussion

Chemistry, composition and characterization of the ligands

The sulfonamide derived ligands (**L**$_1$)-(**L**$_6$) were prepared as shown in Figure 1. All the ligands were only soluble in DMF, DMSO and dioxane. The composition of the ligands is consistent with the

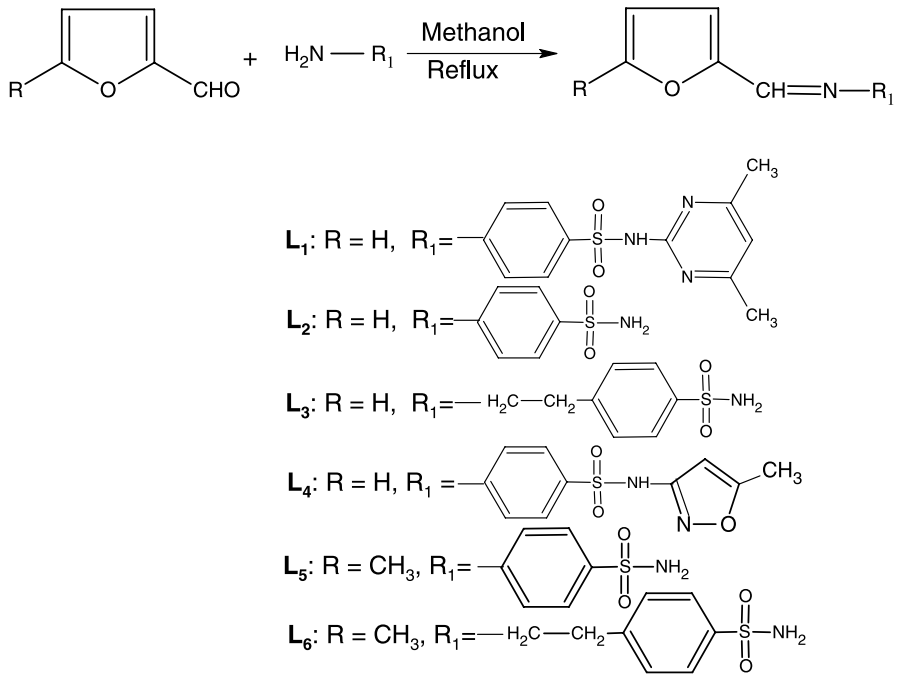

Figure 1. Scheme for the preparation of ligands.

microanalytical data. The ^{1}H NMR spectral data along with assignments is given in the experimental which reveals the appearance [23] of the azomethine proton (—CH═N) signal at 7.13 ppm. This is further supported [24] by the appearance of a band for ν(C══N) (azomethine) at 1590 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 (**L$_1$**)-(**L$_6$**) were prepared according to the following equation.

$$MCl_2 + 2\,\text{Ligand}\,(\mathbf{L}_1) - (\mathbf{L}_6) \rightarrow [M(L)_2Cl_2] \quad (1)$$
$$L{=}(\mathbf{L}_1) - (\mathbf{L}_6)$$

Some physical properties such as melting points and % yields are given in Table I.

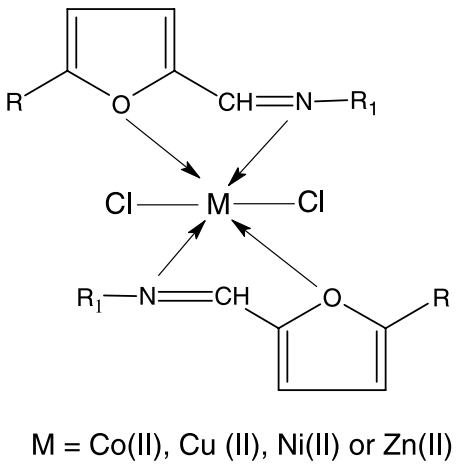

Figure 2. Structure of the metal (II) complexes.

Conductance and magnetic susceptibility measurements

The molar conductance values (in DMF) fall within the range 12–18 Ω^{-1} cm^2 mol^{-1} for all complexes, showing their non-electrolytic [25] nature. This in turn, suggests that the chloride ions are coordinated with the metal ions. The room temperature magnetic moment values of the complexes are given in Table I. The observed magnetic moment (4.89–4.92 B.M.) is consistent with half-spin octahedral cobalt (II) complexes. The magnetic moment values (1.72–1.94 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 [26]. The measured values (3.14–3.26 B.M.) for the nickel (II) complexes suggest [27] octahedral geometry for these complexes. The zinc (II) complexes were found to be diamagnetic [28] as expected.

IR spectra

The important IR spectral bands of the ligands and its metal complexes are given in the Experimental and in

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

No		M.P (°C)	Yield (%)	B.M (μ_{eff})	IR (cm^{-1})	λmax (cm^{-1})	Calc. (Found) % C	H	N
1.	$[Co(L_1)Cl_2]$ [842.64] $C_{34}H_{32}CoCl_2N_8O_6S_2$	243–245	75	4.89	3230 (NH), 1565 (C═N), 1550 (–N═ring), 1335 (C—O), 1325, 1140 (SO_2), 960 (S—N), 29290 845 (C—S), 425 (M—N), 525 (M—O), 315 (M—Cl)	7285, 17360, 20455, 2045, 29290	48.46 (48.61)	3.83 (2.50)	13.30 (13.13)
2.	$[Cu(L_1)Cl_2]$ [847.25] $C_{34}H_{32}CuCl_2N_8O_6S_2$	247–249	77	1.72	3230 (NH), 1575 (C═N), 1550 (–N═ring), 1330 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 440 (M—N), 520 (M—O), 315 (M—Cl)	14815, 19245, 30235	48.20 (48.44)	3.81 (3.57)	13.23 (13.45)
3.	$[Ni(L_1)Cl_2]$ [842.49] $C_{34}H_{32}NiCl_2N_8O_6S_7$	241–243	75	3.14	3230 (NH), 1570 (C═N), 1550 (–N═ ring), 1355 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—5), 430 (M—N), 535 (M—O), 315 (M—CI)	10465, 15715, 26420, 29905	48.48 (48.81)	3.83 (3.58)	13.30 (13.16)
4.	$[Zn(L_1)Cl_2]$ [849.11] $C_{34}H_{32}ZnCl_2N_8O_6S_2$	245–247	77	Dia	3230 (NH), 1568 (C═N), 1550 (–N═ring), 1350 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 435 (M—N), 545 (M—O), 315 (M—CI)	28445	48.09 (48.33)	3.80 (3.52)	13.20 (13.11)
5.	$[Co(L_2)Cl_2]$ [630.38] $C_{22}H_{20}CoCl_2N_4O_6S_2$	211–213	76	4.92	3230 (NH), 1570 (C═N), 1360 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 440 (M—N), 540 (M—O), 315 (M—CI)	7470, 17510, 20670, 29385	41.92 (41.58)	3.20 (3.56)	8.89 (8.63)
6.	$[Cu(L_2)Cl_2]$ [635.00] $C_{22}H_{20}CuCl_2N_4O_6S_2$	205–207	75	1.94	3230 (NH), 1565 (C═N), 1334 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 425 (M—N), 535 (M—O), 315 (M—Cl)	15150, 19415, 30310	41.61 (41.87)	3.17 (3.48)	8.82 (8.56)
7.	$[Ni(L_2)Cl_2]$ [630.14] $C_{22}H_{20}NiCl_2N_4O_6S_2$	209–211	76	3.26	3230 (NH), 1572 (C═N), 1345 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 435 (M—N), 530 (M—O), 315 (M—Cl)	10515, 15860, 26575, 30215	41.93 (42.26)	3.20 (3.12)	8.89 (8.58)
8.	$[Zn(L_2)Cl_2]$ [636.86] $C_{22}H_{20}ZnCl_2N_4O_6S_2$	203–205	75	Dia	3230 (NH), 1570 (C═N), 1335 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 430 (M—N), 530 (M—O), 315 (M—CI)	29130	41.49 (41.61)	3.16 (3.48)	8.80 (8.69)
9.	$[Co(L_3)Cl_2]$ [686.49] $C_{26}H_{28}CoCl_2N_4O_6S_2$	192–194	75	4.90	3230 (NH), 1565 (C═N) 1330 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 440 (M—N), 510 (M—O), 315 (M—CI)	7355, 17480, 20555, 29315	45.49 (45.37)	4.11 (4.37)	8.16 (8.32)
10.	$[Cu(L_3)Cl_2]$ [691.10] $C_{26}H_{28}CuCl_2N_4O_6S_2$	188–190	77	1.85	3230 (NH), 1572 (C═N) 1330 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 430 (M—N), 525 (M—O), 315 (M—CI)	14945, 19270, 30275	45.18 (45.33)	4.08 (3.26)	8.11 (8.36)
11.	$[Ni(L_3)Cl_2]$ [686.25] $C_{26}H_{28}NiCl_2N_4O_6S_2$	197–199	75	3.18	3230 (NH), 1569 (C═N) 1360 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 425 (M—N), 520 (M—O), 315 (M—CI)	10490, 15785, 26555, 30110	45.50 (45.41)	4.11 (4.26)	8.16 (7.94)
12.	$[Zn(L_3)Cl_2]$ [692.97] $C_{26}H_{28}ZnCl_2N_4O_6S_2$	190–192	78	Dia	3230 (NH), 1565 (C═N) 1350 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 430 (M—N), 515 (M—O), 315 (M—CI)	28535	45.06 (45.23)	4.07 (4.33)	8.08 (8.24)

Table I – *continued*

No		M.P (°C)	Yield (%)	B.M (μ_{eff})	IR (cm^{-1})	λmax (cm^{-1})	Calc. (Found) % C	H	N
13.	$[Co(L_4)Cl_2]$ [792.53] $C_{30}H_{26}CoCl_2N_6O_8\ S_2$	247–249	76	4.92	3230 (NH), 1570 (C═N) 1550 (–N═ring), 1325 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 440 (M—N), 530 (M—O), 315 (M—CI)	73910, 17415, 20620, 29380	45.46 (45.28)	3.31 (3.19)	10.60 (10.73)
14.	$[Cu(L_4)Cl_2]$ [797.14] $C_{30}H_{26}CuCl_2N_6O_8\ S_2$	240–242	75	1.91	3230 (NH), 1575 (C═N) , 1550 (–N═ring), 1360, (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 440 (M—N), 540 (M—O), 315 (M—CI)	15115, 19410, 30295	45.20 (45.34)	3.39 (3.57)	10.54 (10.37)
15.	$[Ni(L_4)Cl_2]$ [792.29] $C_{30}H_{26}NiCl_2N_6O_8\ S_2$	239–24	76	3.20	3230 (NH), 1565 (C═N) , 1550 (–N═ring), 1345, (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 435 (M—N), 535 (M—O), 315 (M—CI)	10475, 15780, 26510, 29915	45.48 (45.52)	3.31 (3.23)	10.61 (10.93)
16.	$[Zn(L_4)Cl_2]$ [799.01] $C_{30}H_{26}ZnCl_2N_6O_8\ S_2$	243–245	75	Dia	3230 (NH), 1575 (C═N), 1550 (–N═ring), 1340, (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 430 (M—N), 535 (M—O), 315 (M—CI)	28730	45.10 (45.34)	3.28 (3.55)	10.52 (10.37)
17.	$[Co(L_5)Cl_2]$ [658.44] $C_{24}H_{24}CoCl_2N_4O_6S_2$	248–250	76	4.90	3230 (NH), 1569 (C═N), 1350 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 435 (M—N), 530 (M—O), 315 (M—CI)	7390, 17430, 20565, 29310	43.78 (43.56)	3.67 (3.92)	8.51 (8.37)
18.	$[Cu(L_5)Cl_2]$ [663.05] $C_{24}H_{24}CuCl_2N_4O_6S_2$	249–251	75	1.78	3230 (NH), 1565 (C═N), 1355 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 425 (M—N), 540 (M—O), 315 (M—CI)	14970, 19335, 30290	43.47 (43.51)	3.65 (3.48)	8.45 (8.63)
19.	$[Ni(L_5)Cl_2]$ [658.20] $C_{24}H_{24}NiCl_2N_4O_6S_2$	245–247	76	3.19	3230 (NH), 1575 (C═N), 1340 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 435 (M—N), 530 (M—O), 315 (M—CI)	10470, 15845, 26440, 30200	43.79 (43.45)	3.68 (3.72)	8.51 (8.77)
20.	$[Zn(L_5)Cl_2]$ [664.91] $C_{24}H_{24}ZnCl_2N_4O_6S_2$	242–244	75	Dia	3230 (NH), 1570 (C═N), 1340 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 440 (M—N), 535 (M—O), 315 (M—CI)	29111	43.35 (43.49)	3.64 (3.58)	8.43 (8.61)
21.	$[Co(L_6)Cl_2]$ [714.54] $C_{28}H_{32}CoCl_2N_4O_6S_2$	188–190	76	4.89	3230 (NH), 1572 (C═N), 1345 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 425 (M—N), 525 (M—O), 315 (M—CI)	7330, 17445, 20590, 29335	47.06 (47.18)	4.51 (4.73)	7.84 (7.61)
22.	$[Cu(L_6)Cl_2]$ [719.16] $C_{28}H_{32}CuCl_2N_4O_6S_2$	195–197	75	1.88	3230 (NH), 1575 (C═N), 1335 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 430 (M—N), 515 (M—O), 315 (M—CI)	14995, 19310, 30245	47.76 (47.84)	4.48 (4.39)	7.79 (7.81)
23.	$[Ni(L_6)Cl_2]$ [714.30] $C_{28}H_{32}NiCl_2N_4O_6S_2$	185–187	76	3.22	3230 (NH), 1565 (C═N), 1360 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 425 (M—N), 545 (M—O), 315 (M—CI)	10475, 15810, 264995, 30100	47.08 (47.26)	4.52 (4.68)	7.84 (7.97)
24.	$[Zn(L_6)Cl_2]$ [721.02] $C_{28}H_{32}ZnCl_2N_4O_6S_2$	190–192	75	Dia	3230 (NH), 1570 (C═N), 1330 (C—O), 1325, 1140 (SO_2), 960 (S—N), 845 (C—S), 435 (M—N), 510 (M—O), 315 (M—CI)	28915	46.64 (46.93)	4.47 (4.18)	7.77 (7.89)

Table I. All ligands contain four potential donor sites: the furanyl oxygen, the azomethine nitrogen, the sulphonamide oxygens, the sulphonamide nitrogen and/or, in case of ligands $\mathbf{L_1}$ and $\mathbf{L_4}$, the additional pyrimidine nitrogens and isoxazole nitrogen/oxygen. In the IR spectra of the ligands a sharp band observed at 1590 cm^{-1} is assigned [29] to the ν(C═N) mode and a medium sharp band at 1385 cm^{-1} is due to the ν(C—O) stretching of the furanyl 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 15–25 cm^{-1} (1565–1575 cm^{-1}) in all of the complexes. This is further confirmed by the appearance of the new bands at 425–440 cm^{-1} due to the ν(M—N) band [30].

The coordination through the furanyl ring oxygen is revealed by shifting of the C—O band at 1385 cm^{-1} to much lower frequencies (1330–1360 cm^{-1}) in all the complexes as compared to that of the ligands. This is further confirmed by the appearance of the new band at 510–545 cm^{-1} due to ν(M—O) in all the complexes. The bands in the ligand due to ν_{asymm}(-SO_2) and $\nu_{symm}(SO_2)$ appear at 1325 and 1140 cm^{-1}, respectively [31]. 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 [32], in the ligands after complexation. Also, in ligands $\mathbf{L_1}$ and $\mathbf{L_4}$ the band due to the ν(—N═) pyrimidine or isoxazole ring appearing at 1550 cm^{-1} did not show any appreciable change on complexation suggesting that these ring nitrogens in these moieties are not taking part in coordination. A new band appearing at 315 cm^{-1} assigned [33] to the ν(M—Cl) mode in all the metal complexes was, however, indicative that chloride atoms are coordinated with the central metal atom.

^{1}H NMR spectra

^{1}H NMR spectra of the free ligands and their diamagnetic zinc (II) complexes were recorded in DMSO-d_6. The ^{1}H NMR spectral data along with the possible assignments is recorded in the Experimental part. All the protons due to heteroaromatic/aromatic groups were found to be in their expected region [34]. The conclusions drawn from these studies lend further support to the mode of bonding discussed for their IR spectra. The coordination of the azomethine nitrogen is inferred by the downfield shift of the —C*H*═N- proton signal from 7.73 ppm in the ligand to 8.52–8.68 ppm in the complexes. Also, the furanyl protons underwent a downfield shift by 0.5–0.7 ppm due to the increased conjugation [35] and coordination of furanyl oxygen ring to the metal atoms. Furthermore, the number of protons calculated from the integration curves, and those obtained from the values of the expected CHN analyses agreed.

Electronic spectra

The Co(II) complexes exhibited well-resolved, low-energy bands at 7,285–7,470 cm^{-1}, 17,360–17,510 cm^{-1} and a strong high-energy band at 20,455–20,670 cm^{-1} (Table I) 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 [37]. A high intensity band at 29,290–29,385 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 [38] consistency with their octahedral environment.

The electronic spectra of the Cu (II) complexes (Table I) showed two low-energy weak bands at 14,815–15,150 cm^{-1} and 19,245–19,415 cm^{-1} and a strong high-energy band at 30,235–30,310 cm^{-1} and may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively [39]. 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 [40].

The electronic spectra of the Ni (II) complexes showed d-d bands in the region 10,465–10,515, 15,715–15,860 and 26,420–26,575 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 29,905–30,215 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements showed two unpaired electrons per Ni (II) ion suggesting [39] also an octahedral geometry for the Ni (II) complexes. The electronic spectra of the Zn (II) complexes exhibited only a high-intensity band at 28,445–29,130 cm^{-1} and are assigned [40] to a ligand-metal charge transfer.

Biological activity

Antibacterial bioassay. All compounds were tested against six Gram-negative (*E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, S. typhi* and *S. dysenteriae*) and four Gram-positive (*B. cereus, C. diphtheriae, S. aureous* and *S. pyogenes*) bacterial strains (Table II) according to literature protocol [18,19]. The results were compared with those of the standard drug imipenum. All ligands showed moderate to significant activity against all Gram-negative and Gram-positive bacterial strains except against *S. flexneri (b)* that showed a weak activity. Compounds (**1**)–(**24**)

Table II. Antibacterial bioassay (concentration used 1 mg/mL of DMSO).

	Compound (zone of inhibition)																														
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**
Gram-negative																															
(a)	16	18	19	15	17	19	17	18	19	20	18	20	20	22	19	20	19	22	16	16	15	19	18	19	20	22	21	19	20	24	30
(b)	09	07	09	06	08	10	11	11	12	15	10	11	11	15	12	15	14	17	11	12	12	13	10	11	10	14	12	12	11	15	27
(c)	15	17	18	14	16	18	15	18	19	20	16	19	20	22	18	19	20	21	17	18	19	20	18	19	20	22	20	19	19	22	26
(d)	11	15	17	14	17	20	13	16	17	22	17	16	18	20	20	19	20	22	17	18	18	20	20	20	19	22	20	20	21	23	27
Gram-positive																															
(e)	17	15	18	17	17	18	18	18	17	19	19	20	19	22	19	20	20	22	17	18	19	18	19	19	19	20	20	19	22	24	30
(f)	18	17	18	14	15	17	18	19	18	19	19	19	20	22	20	19	22	24	18	17	18	22	19	19	18	21	20	21	20	23	28

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

Table III. 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	19	00	00	25	00	00	00	00	00	00	00	10	00	20	00	23	00	00	00	05	00	00	00	00	00	27	00	28	A
(b)	00	07	00	00	00	00	00	00	00	00	00	20	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	15	B
(c)	15	00	00	00	00	18	00	00	00	18	00	09	00	00	00	10	00	00	00	00	00	28	00	00	00	00	00	30	00	00	C
(d)	10	00	00	15	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	00	D
(e)	00	15	00	00	00	00	00	00	00	00	00	20	00	00	27	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	E
(f)	00	00	00	25	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	28	00	28	00	00	00	00	00	00	00	00	F

(a) = T. longifucus, (b) = C. Albicans, (c) = A. flavus, (d) = M. canis, (e) = F. Solani, (f) = C. glaberata.
SD = Standard Drugs MIC μg/mL; A = Miconazole (70 μg/mL: 1.6822×10^{-7} M), B = Miconazole (110.8 μg/mL: 2.6626×10^{-7} M), C = Amphotericin B (20 μg/mL: 2.1642×10^{-8} M), D = Miconazole (98.4 μg/mL: 2.3647×10^{-7} M), E = Miconazole (73.25 μg/mL: 1.7603×10^{-7} M), F = Miconazole (110.8 μg/mL: 2.66266×10^{-7} M).

Table IV. Minimum inhibitory concentration (M) of the selected compounds (**4**), (**8**), (**12**), (**20**) and (**24**) against selected bacteria.

No.	(**4**)	(**8**)	(**12**)	(**20**)	(**24**)
Gram-negative					
E.coli	1.1777×10^{-8}	3.9255×10^{-8}	1.4430×10^{-8}	1.5039×10^{-8}	1.3869×10^{-8}
P. aeruginosa	2.9442×10^{-8}	1.5702×10^{-7}	3.6076×10^{-8}	7.5198×10^{-8}	1.3869×10^{-8}
S. typhi	1.1777×10^{-8}	7.8510×10^{-8}	7.2153×10^{-8}	3.7599×10^{-8}	1.3869×10^{-8}
Gram-positive					
S. aureus	1.1777×10^{-7}	7.8510×10^{-8}	1.4430×10^{-7}	7.5198×10^{-8}	1.3869×10^{-8}
B. subtilis	1.1777×10^{-7}	1.5702×10^{-7}	1.4430×10^{-7}	1.5039×10^{-7}	1.3869×10^{-7}

exhibited overall a significant activity against *E. coli, P. aeruginosa, S. typhi, S. aureus* and *B. subtilis*. However a moderate activity was observed by compound (**1**) against *P. aeruginosa* and *S. typhi*. The Zinc (II) complexes of all the ligands were 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 increased from methyl to ethyl in compounds (**9**)–(**12**) and (**21**)–(**24**) the bactericidal activity was increased as compared to the other compounds (**1**)–(**8**) and (**13**)–(**20**) where there were no methyl or ethyl carbon chain present. The structure-activity relationship studies (SAR) suggested that compounds having a free amino group on one side of the ligand as well as its metal chelates were found to exhibit more activity.

Antifungal bioassay. The antifungal screening of all compounds was carried out against *T. longifusus, C. albican, A. flavus, M. canis, F. solani* and *C. glaberate* fungal strains according to the literature protocol [20]. The results of inhibition (in %) were compared with the inhibition by the standard drugs miconazole and amphotericin B. These results in Table III indicate that compounds (**22**) and (**24**) showed significant activity against (*a*), (**6**) against (*b*), (**16**) and (**22**) against (*c*), (**14**) and (**16**) against (*d*), (**9**) against (*e*) and, compounds (**14**) and (**16**) against (*f*) fungal strains.

Minimum inhibitory concentration (MIC) for antibacterial activity. The preliminary antibacterial screening showed that compounds (**4**),(**8**), (**12**), (**20**) and (**24**) were the most active ones (above 80%). These compounds were therefore, selected for antibacterial minimum inhibitory concentration (MIC) studies (Table IV).

Cytotoxic bioassay. All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer et al. [21]. From the data recorded in Table V, it is evident that only two compounds, (**6**), (**10**) and (**23**) displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive in this assay. Compound (**6**) showed activity (LD_{50}=117.7 M) in the present series of compounds, whereas the other active compounds (**10**) and (**23**) of the series demonstrated activity, LD_{50}=125.6 μg/ml and LD_{50}=137.8 M, respectively.

This enhancement in the activity of ($\mathbf{L^1}$-$\mathbf{L^6}$) may be rationalized on the basis of their structures. It has been

Table V. Brine shrimp bioassay data of the ligands ($\mathbf{L_1}$)-($\mathbf{L_6}$) and their metal (II) complexes (**1**)–(**24**).

Compound	LD_{50} (M)	Compound	LD_{50} M
$\mathbf{L_1}$	$>2.8059 \times 10^{-3}$	**10**	1.8173×10^{-4}
$\mathbf{L_2}$	$>3.9956 \times 10^{-3}$	**11**	$>1.4571 \times 10^{-3}$
$\mathbf{L_3}$	$>3.5929 \times 10^{-3}$	**12**	$>1.4430 \times 10^{-3}$
$\mathbf{L_4}$	$>3.0180 \times 10^{-3}$	**13**	$>1.2617 \times 10^{-3}$
$\mathbf{L_5}$	$>3.7835 \times 10^{-3}$	**14**	$>1.2544 \times 10^{-3}$
$\mathbf{L_6}$	$>3.4205 \times 10^{-3}$	**15**	$>1.2621 \times 10^{-3}$
1	$>1.1867 \times 10^{-3}$	**16**	$>1.2515 \times 10^{-3}$
2	$>1.1862 \times 10^{-3}$	**17**	$>1.5187 \times 10^{-3}$
3	$>1.1869 \times 10^{-3}$	**18**	$>1.5081 \times 10^{-3}$
4	$>1.1777 \times 10^{-3}$	**19**	$>1.5192 \times 10^{-3}$
5	$>1.5863 \times 10^{-3}$	**20**	$>1.5039 \times 10^{-3}$
6	1.8535×10^{-4}	**21**	$>1.3995 \times 10^{-3}$
7	$>1.5869 \times 10^{-3}$	**22**	$>1.3905 \times 10^{-3}$
8	$>1.5702 \times 10^{-3}$	**23**	1.9291×10^{-4}
9	$>1.4566 \times 10^{-3}$	**24**	$>1.3869 \times 10^{-3}$

suggested that chelation/coordination reduces the polarity of the metal ion [41–45] because of the partial sharing of its positive charge with the donor groups and possibly the π-electron delocalisation 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 [46–50] 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 [51–55] extensive biological activities that may be responsible for the increase of 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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