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Metal-based sulfonamides: Their preparation, characterization and *in-vitro* antibacterial, antifungal & cytotoxic properties. X-ray structure of 4-[(2-hydroxybenzylidene) amino] benzenesulfonamide

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Abstract

Synthesis, characterization and biological studies of Schiff base-derived sulfonamides and their Co (II), Cu (II), Ni (II) and Zn (II) complexes have been reported and screened for *in-vitro* antibacterial activity against six Gram-negative; *E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis, S. typhi* and *S. dysenteriae* and four Gram-positive; *B. cereus, C. diphtheriae, S. aureus* and *S. pyogenes* bacterial strains and for *in-vitro* antifungal activity against *T. longifusus, C. albicans, A. flavus, M. canis, F. solani,* and *C. glaberata.* All compounds showed moderate to significant antibacterial activity, however, the zinc (II) complexes were found to be more active. Some of the compounds also showed significant antifungal activity against various fungal strains. Only compounds **(6)** and **(10)** displayed potent cytotoxic activity with $LD_{50} = 4.644 \times 10^{-4}$ and 4.106×10^{-4} moles/mL respectively, against *Artemia salina*. The X-ray structure of 4-[(2-hydroxybenzylidene)amino]benzenesulfonamide is also reported.

Keywords: *Sulfonamides, Metal complexes, Antibacterial, Antifungal, Cytotoxicity, X-ray structure*

Introduction

A number of sulfonamides and their derived compounds have been reported for their antibacterial [1–3], antitumor [4], anti-carbonic anhydrase [5–10], diuretic [11], hypoglycaemic [12], antithyroid [13] and protease inhibitory activity [14,15]. Due to their significant pharmacology applications and wide-spread use in medicine, these compounds have gained attention in bio-inorganic and metal-based drug chemistry. Metal-based pharmacological properties of sulfonamides were surprisingly observed when they were administered as metal chelates initially coming to light when the silver (I) complex of sulfanilamide was prepared [16] and tested for biological activity. Then other metal complexes of substituted sulfanilamides were subsequently synthesized and investigated for enhanced biological activities in detail [17].

The growing interest in metal-sulfonamides drug chemistry also attracted the attention of the author and forced to commence a program [18–28] in meticulously designing and exploring this potential area of research. With this idea, a series of such metal-based sulfonamides and sulphonamide-derived compounds having potential therapeutic properties have been previasly reported. In this context, some now sulfonamides and their metal-based compounds have been reported with their potential bactericidal and fungicidal agents against various bacterial and fungal resistant species, whose increasing incidence is emerging globally as a major problem in drug therapy.

Materials and methods

The solvents used were of analytical grade; 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. *In-vitro* antibacterial, antifungal and cytotoxic properties

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were determined at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Preparation of Schiff's bases (L_1–L_5)

To an ethanolic (25 mL) solution of the respective sulfonamide (0.007 moles), an ethanol solution of the relevant aldehyde (0.007 moles) was added with stirring. The solution was refluxed for 3 h. After cooling to room temperature, the obtained solution was filtered and left overnight at room temperature, which led to the formation of a crystalline product.

This crystalline product was filtered and recrystallized from hot ethanol to give the orange-red desired product. Purification was checked by TLC, which indicated a single spot. All other Schiff's bases **(L_1–L_5)** were prepared following the same method.

Preparation of metal complexes (1)–(12)

To a hot ethanol (25 mL) solution of the respective sulfonamide (0.02 moles), an ethanol solution of the corresponding metal (II) salt (0.01 moles) was added. The reactant mixture was refluxed for 2 h and the reaction was monitored through TLC. After completion of the reaction, precipitates appeared on cooling in an ice-bath, which were filtered and recrystallized from aqueous-ethanol (1: 3) affording TLC-pure products in good yield.

Only one compound, 4-[(2-hydroxybenzylidene) amino] benzenesulfonamide produced sufficiently good crystals for X-ray structural determination. All other compounds were either amorphous powders or tiny crystals which were unsuitable for X-ray studies.

*4-[(4-Hydroxy-3-methoxybenzylidene)amino]benzenesulfonamide (**L_1**)*. Yield 77%; m.p. 199 °C; IR (KBr, cm^{-1}): 3455 (NH_2), 3325 (OH), 1590 (HC═N), 1315, 1110 (S═O), 960, 845; ^{1}H NMR (DMSO-d_6, δ, ppm): 2.54 (s, 3H, methoxy), 7.35–7.54 (m, 4H, benzene), 7.68–7.77 (m, 3H, benzylidene), 7.85 (br s, 2H, SO_2NH_2), 7.96 (s, 1H, CH═N), 10.85 (s, 1H, OH); Anal. Calcd. for $C_{14}H_{14}N_2O_4S$ (306.33): C, 54.89; H, 4.61; N, 9.14. Found: C, 55.12; H, 4.95; N, 9.01%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 3.11 (s, 3H, methoxy), 7.73–7.85 (m, 4H, benzene), 7.91–7.98 (m, 3H, benzylidene), 8.37 (br s, 2H, SO_2NH_2), 8.64 (s, 1H, CH═N), 10.97 (s, 1H, OH).

*4-{2-[(4-Hydroxy-3-methoxybenzylidene)amino]ethyl}benzenesulfonamide (**L_2**)*. Yield 73%; m.p. 157 °C; IR (KBr, cm^{-1}): 3455 (NH_2), 3325 (OH), 1590 (HC═N), 1315, 1110 (S═O), 960, 845; ^{1}H NMR (DMSO-d_6, δ, ppm): 2.54 (s, 3H, methoxy), 5.46–5.71(t, 2H, CH_2), 6.42 (t, 2H, NCH_2), 7.31–7.53 (m, 4H, benzene), 7.68–7.77 (m, 3H, benzylidene), 7.85 (br s, 2H, SO_2NH_2), 7.96 (s, 1H, CH═N), 10.85 (s, 1H, OH); Anal. Calcd. for $C_{16}H_{18}N_2O_4S$ (334.39): C, 57.47; H, 5.43; N, 8.38. Found: C, 57.12; H, 5.65; N, 8.64%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 3.13 (s, 3H, methoxy), 6.18–6.27 (t, 2H, CH_2), 6.84 (t, 2H, NCH_2), 7.74–7.81 (m, 4H, benzene), 7.92–7.99 (m, 3H, benzylidene), 8.35 (br s, 2H, SO_2NH_2), 8.64 (s, 1H, CH═N), 10.98 (s, 1H, OH).

*4-[(2-Hydroxybenzylidene)amino]benzenesulfonamide (**L_3**)*. Yield 76%; m.p. 208°C; IR (KBr, cm^{-1}): 3455 (NH_2), 3325 (OH), 1590 (HC═N), 1315, 1110 (S═O), 1385 (C–O), 960, 845; ^{1}H NMR (DMSO-d_6, δ, ppm): 7.35–7.54 (m, 4H, benzene), 7.68–7.77 (m, 4H, benzylidene), 7.85 (br s, 2H, SO_2NH_2), 7.96 (s, 1H, CH═N), 10.85 (s, 1H, OH); Anal. Calcd. for $C_{13}H_{12}N_2O_3S$ (276.31): C, 56.51; H, 4.38; N, 10.14. Found: C, 56.82; H, 4.75; N, 10.37%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 7.74–7.82 (m, 4H, benzene), 7.92–7.98 (m, 4H, benzylidene), 8.37 (br s, 2H, SO_2NH_2), 8.65 (s, 1H, CH═N).

*4-{[(2-Hydroxybenzylidene)amino]methyl}benzenesulfonamide (**L_4**)*. Yield 66%; m.p. 209 °C; IR (KBr, cm^{-1}): 3455 (NH_2), 3325 (OH), 1590 (HC═N), 1315, 1110 (S═O), 1385 (C–O), 960, 845; ^{1}H NMR (DMSO-d_6, δ, ppm): 6.42 (t, 2H, NCH_2), 7.31–7.53 (m, 4H, benzene), 7.68–7.77 (m, 4H, benzylidene), 7.85 (br s, 2H, SO_2NH_2), 7.96 (s, 1H, CH═N), 10.85 (s, 1H, OH); Anal. Calcd. for $C_{14}H_{14}N_2O_3S$ (290.33): C, 57.92; H, 4.86; N, 9.65. Found: C, 58.22; H, 4.58; N, 9.82%. ^{1}H NMR of Zn (II) complex (DMSO-d_6, δ, ppm): 7.38 (t, 2H, NCH_2), 7.74–7.83 (m, 4H, benzene), 7.92–7.97 (m, 4H, benzylidene), 8.36 (br s, 2H, SO_2NH_2), 8.65 (s, 1H, CH═N).

*4-{2-[(2-Hydroxybenzylidene)amino]ethyl}benzenesulfonamide (**L_5**)*. Yield 81%; m.p. 137°C; IR (KBr, cm^{-1}): 3455 (NH_2), 3325 (OH), 1590 (HC═N), 1315, 1110 (S═O), 1385 (C–O), 960, 845; ^{1}H NMR (DMSO-d_6, δ, ppm): 5.46–5.71 (t, 2H, CH_2), 6.42 (t, 2H, NCH_2), 7.31–7.53 (m, 4H, benzene), 7.68–7.77 (m, 3H, benzylidene), 7.85 (br s, 2H, SO_2NH_2), 7.96 (s, 1H, CH═N), 10.85 (s, 1H, OH); Anal. Calcd. for $C_{15}H_{16}N_2O_3S$ (304.36): C, 59.19; H, 5.30; N, 9.20. Found: C, 59.38; H, 5.42; N, 9.48%. 1H NMR of Zn (II) complex (DMSO-d6, δ, ppm): 6.17–6.27 (t, 2H, CH_2), 6.85 (t, 2H, NCH_2), 7.74–7.81 (m, 4H, benzene), 7.92–7.98 (m, 3H, benzylidene), 8.35 (br s, 2H, SO_2NH_2), 8.64 (s, 1H, CH═N).

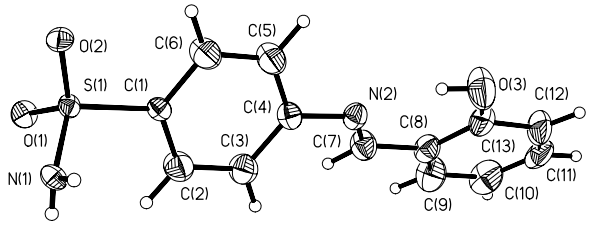


Figure 1. ORTEP diagram of a single molecule found in the asymmetric unit.

X-ray crystallography

X-ray structure and atom labeling scheme of the novel 4-[(2-hydroxybenzene)- amino]benzenesulfonamide **(L_3)** is presented in Figure 1, and crystal and refinement data of a yellow needle crystal of $C_{13}H_{12}N_2O_3S$ having approximate dimensions of 0.20 × 0.20 × 0.20 mm are presented in Tables VI and VII.

Antibacterial bioassay (in-vitro)

All the synthesized compounds **(L_1)–(L_5)** and metal (II) complexes **(1)–(12)** were screened *in-vitro* for their antibacterial activity against six Gram-negative (*E. coli, K. pneumonia, P. aeruginosa, P. mirabilis, S. typhi* and *S. dysenteriae*) and four Gram-positive (*B. cereus, C. diphtheriae, S. aureus and S. pyogenes*) bacterial strains by the agar-well diffusion method [29,30]. The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Two to eight h old bacterial inocula containing approximately 10^4–10^6 colony-forming units (CFU/mL) were spread on the surface of the nutrient agar with the help of a sterile cotton swab. The recommended concentration of the test sample (1 mg/mL in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenum, served as negative and positive controls, respectively.

The plates were incubated immediately at 37°C for 24 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains.

Antifungal Activity (in-vitro)

Antifungal activities of all compounds were studied against six fungal cultures. Sabouraud dextrose agar (oxoid, Hampshire, England) was seeded with 10^5 (cfu) mL^{-1} fungal spore suspensions and transferred to petri plates. Discs soaked in 20 ml (200 μg/mL in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32°C for seven days. The results were recorded [31] as % inhibition and compared with standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration (MIC)

Compounds showing high antibacterial activity (over 80%) were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion technique by preparing discs containing 10, 25, 50 and 100 μg/mL of the compounds and applying the protocol [32].

Cytotoxicity (in-vitro)

Brine shrimp (Artemia salina leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the minor 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 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 two days, when the shrimp larvae were ready, 1 mL of sea water and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h the number of survivors was counted. Data were analyzed by a Finney computer program to determine the LD_{50} values [33,34].

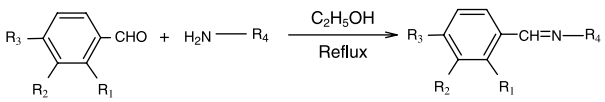
Result and discussion

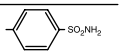
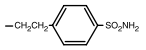
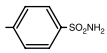
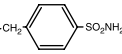
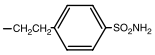
Chemistry, composition and characterization of the ligands

The sulfonamide derived ligands **(L_1)–(L_5)** were prepared as shown in Scheme 1. All ligands were only soluble in hot ethanol, DMF and DMSO. The composition of the ligands is consistent with their microanalytical data.

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

The metal (II) complexes **(1)–(12)** of the ligands **(L_3)–(L_5)** were prepared according to the following



No.	R_1	R_2	R_3	R_4
L_1:	H	OCH_3	OH	
L_2:	H	OCH_3	OH	
L_3:	OH	H	H	
L_4:	OH	H	H	
L_5:	OH	H	H	

Scheme 1. Preparation of Ligands.

equations:

$$MCI_2 + 2\text{Ligand (L)} \rightarrow [M(L)_2(OH_2)_2] \qquad L = (\boldsymbol{L}_3) - (\boldsymbol{L}_5) \qquad (1)$$

The ligands **(L_1)** and **(L_2)** however, could not form metal complexes because of the presence of the (OH) group at a position which is not able to donate its lone pair towards the metal atom to form a coordination compound. Some physical properties such as melting points and % yields are given in Table I. The proposed structure of the compexes is shown in Figure 2.

Conductance and magnetic susceptibility measurements. The molar conductance values (in DMF) for complexes **(1)**–**(12)** fall within the range 86–92 Ω^{-1} cm^2 mol^{-1}, showing their non-electrolytic nature[35].

The room temperature magnetic moment values of the complexes are given in Table I. The observed magnetic moment (4.76–4.88 B.M.) is consistent with half-spin octahedral cobalt (II) complexes. The magnetic moment values (1.67–1.81 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 [36]. The measured values (3.25–3.30 B.M.) for the nickel (II) complexes suggest [37] octahedral geometry for these complexes. The zinc (II) complexes were found to be diamagnetic as expected.

IR spectra. The important IR spectral bands of the ligands and their metal complexes are given in the Experimental and in Table I. All ligands contain four potential donor sites: the benzylidene hydroxyl oxygen, the azomethine nitrogen, the sulfonamide oxygen and the sulfonamide nitrogen. In the IR spectra of the ligands a broad band observed at 3325 cm^{-1} and a sharp band at 1590 cm^{-1} are assigned [38] to the ν(OH) and (C=N) modes 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 its metal complexes. This is further supported by the appearance of the new bands at 425–440 cm^{-1} due to the ν(M–N) band [39].

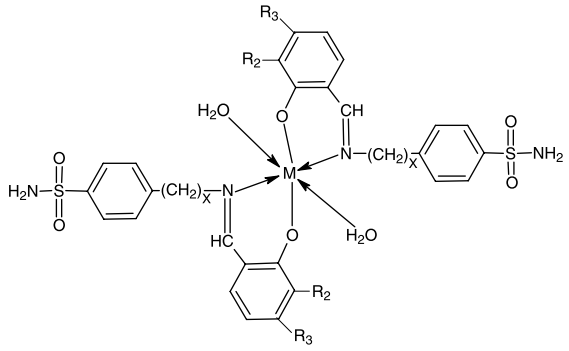
The coordination through the hydroxyl oxygen is revealed only in ligands **(L_3)–(L_5)** by disappearance of the mode at 3325 cm^{-1} and appearance of a new band at 1385 cm^{-1} due to the C–O mode. This is further confirmed by the appearance of the new band at 510–545 cm^{-1} due to ν(M–O) in the metal complexes of ligands **(L_3)**–**(L_5)**. The disappearance of a band assigned to the hydroxyl group in ligands **(L_1)** and **(L_2)** was not observed indicating that the hydroxyl group of these ligands does not participate in coordination. This is due to the fact that the hydroxyl group in ligands **(L_1)** and **(L_2)** can not involve in participation with the metal atom whereas in the case of ligands **(L_3)**–**(L_5)**, the hydroxyl group readily involves itself in the coordination process. The bands in the ligand due to $\nu_{asymm}(SO_2)$ and $\nu_{symm}(SO_2)$ appear at 1315 and 1140 cm^{-1}, respectively [40]. 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 [41,42], in the ligands after complexation.

^1H NMR spectra. ^{1}H NMR spectra of the free ligands and their diamagnetic zinc (II) complexes were recorded in DMSO-d_6. The ^{1}H NMR spectral data along with the possible assignments is recorded in the Experimental. All the protons due to heteroaromatic/aromatic groups were found to be in their expected region [43]. The conclusions drawn from these studies lend further support to the mode of bonding discussed in their IR spectra. The coordination of the azomethine nitrogen is inferred by the downfield shift of the –CH=N- proton signal from 7.96 ppm in the ligand to 8.52–8.68 ppm in the complexes. The hydroxyl proton at 10.85 ppm in the spectra of Zn (II) complexes of ligands **(L_3)**–**(L_5)** disappeared indicating deprotonation and coordination of the oxygen with the metal ion whereas this hydroxyl proton in the spectra of Zn (II) complexes of ligands **(L_1)** and **(L_2)** did not disappear suggesting their non-coordination. All other protons underwent a downfield shift by 0.3–0.6 ppm due to the increased conjugation [44] and coordination with the metal atom.

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

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. $[Co(L_3)(H_2O)_2]$ [645.57] $C_{26}H_{26}N_4O_8S_2$ Co	211–213	83	4.80	3455 (NH_2), 1575 (C═N), 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 440 (M–N) (M–O)	7290, 17420, 20470, 29310	48.37(48.62)	4.06 (4.33)	8.68 (8.42)
2. $[Cu(L_3)(H_2O)_2]$ [650.19] $C_{26}H_{26}CuN_4O_8S_2$	206–208	81	1.72	3455 (NH_2), 1565 (C═N), 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N) 845 (C–S), 425 (M–N) 510, (M–O)	14825, 19165 30355	48.03 (48.47)	4.03 (3.87)	8.62 (8.84)
3.$[Ni(L_3)(H_2O)_2]$ [645.33] $C_{26}H_{26}NiN_4O_8S_2$	216–218	82	3.30	3455 (NH_2), 1570 (C═N), 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 430 (M–N), 545 (M–O)	10435, 19165, 30355	48.03 (48.47)	4.03 (3.87)	8.62 (8.84)
4. $[Zn(L_3)(H_2O)_2]$ [652.03] $C_{26}H_{26}ZnN_4O_8S_2$	212–214	80	Dia	3455 (NH_2), 1568 (C═N), 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 437 (M–N), 528 (M–O)	28585	47.89 (47.69)	4.02 (4.46)	8.59 (8.73)
5. $[Co(L_4)(H_2O)_2]$ [673.63] $C_{28}H_{30}CoN_4O_8S_2$	204–206	82	4.88	3455 (NH_2), 1565 (C═N), 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 425 (M–N), 525 (M–O)	7470, 17510, 20670, 29385	49.92 (50.21	4.49 (4.18)	8.32 (8.65)
6. $[Cu(L_4)(H_2O)_2]$ [678.24] $C_{28}H_{30}CuN_4O_8S_2$	206–208	84	1.67	3455 (NH_2), 1575 (C═N), 1385 (C–O), 1315, 1140, (SO_2), 960 (S–N), 845 (C–S), 428 (M–N), 520 (M–O)	15155, 19315, 30380	49.59 (49.33)	4.46 (4.69)	8.26 (8.43)
7. $[Ni(L_4)(H_2O)_2]$ [673.38] $C_{28}H_{30}NiN_4O_8S_2$	209–211	81	3.25	3455 (NH_2), 1570 (C═N) 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 432 (M–N), 533 (M–O)	10485, 15865, 26675, 30225	49.94 (50.37)	4.49 (4.16)	8.32 (8.16)
8. $[Zn(L_4)(H_2O)_2]$ [680.08] $C_{28}H_{30}ZnN_4O_8S_2$	214–216	82	Dia	3455 (NH_2), 1568 (C═N), 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 425 (M–N), 545 (M–O)	29140	49.45 (49.81)	4.45 (4.77)	8.24 (8.08)
9. $[Co(L_5)(H_2O)_2]$ [701.68] $C_{30}H_{34}CoN_4O_8S_2$	212–214	83	4.76	3455 (NH_2), 1565 (C═N), 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 435 (M–N), 510 (M–O)	7285, 17360, 20455, 29290	51.35 (51.16)	4.88 (4.58)	7.98 (8.24
10. $[Cu(L_5)(H_2O)_2]$ [706.29] $C_{30}H_{34}CuN_4O_8S_2$	210–212	81	1.81	3455 (NH_2), 1567(C═N) 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 430 (M–N), 520 (M–O)	14725, 19140, 30335	51.02 (51.42)	4.85 (4.67)	7.93 (7.68)
11. $[Ni(L_5)(H_2O)_2]$ [701.44] $C_{30}H_{34}NiN_4O_8S_2$	218–220	82	3.27	3455 (NH_2), 1570 (C═N), 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 440 (M–N), 535 (M–O)	10355, 15610, 26325, 29850	51.37 (51.67)	4.89 (4.52)	7.99 (8.28
12. $[Zn(L_5)(H_2O)_2]$ [708.14] $C_{30}H_{34}ZnN_4O_8S_2$	207–209	83	Dia	3455 (NH_2), 1568 (C═N), 1385 (C–O), 1315, 1140 (SO_2), 960 (S–N), 845 (C–S), 439 (M–N), 523 (M–O)	28540	50.88 (50.96)	4.84 (4.52)	7.91 (8.22)



M=Co (II), Cu (II), Ni (II) or Zn (II); x= 0, 1 or 2

Figure 2. Proposed structure of the metal complex.

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 $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ in an 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 [45] consistency with their octahedral environment.

The electronic spectra of the Cu (II) complexes (Table I) showed two low-energy weak bands at 14,725–15,155 cm^{-1} and 19,140–19,315 cm^{-1} and a strong high-energy band at 30,335–30,380 cm^{-1} which 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 a 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 [37].

The electronic spectra of the Ni (II) complexes showed d-d bands in the region 10,355–10,485, 15,610–15,865 and 26,325–26,675 cm^{-1}. These are assigned [47] to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$, respectively, consistent with their well-defined octahedral configuration. The band at 29,850–30,225 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements showed two unpaired electrons per Ni (II) ion suggesting [46] also an octahedral geometry for the Ni (II) complexes. The electronic spectra of the Zn (II) complexes exhibited only a high-intensity band at 28,540–29,140 cm^{-1} and are assigned [47] to a ligand-metal charge transfer.

Biological activity

*Antibacterial bioassay (*in-vitro*)*. 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. aureus* and *S. pyogenes*) bacterial strains (Table II) according to literature protocol [29,30].

The results were compared with those of the standard drug imipenum (Figure 3). All ligands showed moderate to significant activity against all Gram-negative and Gram-positive bacterial strains except the activity of all compounds against strain (b) where moderate to significant activity was not observed. Compounds **(2)–(12)** exhibited overall a significant activity (above 80%) against *(a)*. 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 chains in the ligands and their respective metal chelates had an impact on the bactericidal activity. As the carbon chain increased from methylene to ethylene in compounds **(5)**–**(12)** the bactericidal activity was increased as compared to

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

	Compound																	
Bacteria	**L_1**	**L_2**	**L_3**	**L_4**	**L_5**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**SD**
Gram-negative																		
(a)	16	18	20	21	23	23	24	24	26	24	25	25	26	27	26	27	28	30
(b)	10	08	09	10	11	10	11	10	12	10	11	11	13	14	13	15	15	27
(c)	14	15	15	16	18	17	17	18	19	20	18	19	20	19	18	19	21	26
(d)	13	15	16	16	17	18	17	18	19	18	17	18	20	19	19	20	22	27
(e)	17	15	18	19	19	19	18	19	20	19	20	20	21	22	20	22	25	30
(f)	13	12	13	15	16	16	15	17	18	17	17	18	20	18	19	21	26	28
Gram-positive																		
(g)	15	17	18	19	20	19	18	19	20	20	21	21	22	21	20	22	23	26
(h)	11	15	17	18	20	19	18	19	20	19	19	20	21	20	20	20	22	27
(j)	17	15	16	17	18	18	18	18	19	18	19	20	20	21	20	22	24	30
(k)	16	17	16	17	18	17	18	18	20	18	20	20	21	19	20	21	24	28

(a) = E. coli, (b) = K. pneumoniae, (c) = P. aeruginosa, (d) = P. mirabilis, (e) = S. typhi, (f) = S. dysenteriae, (g) = B. cereus, (h) = C. diphtheriae, (j) = S. aureus and (k) = S. pyogenes. < 10: weak; > 10: moderate; > 16: Significant. SD = Standard Drug (Imipenum)

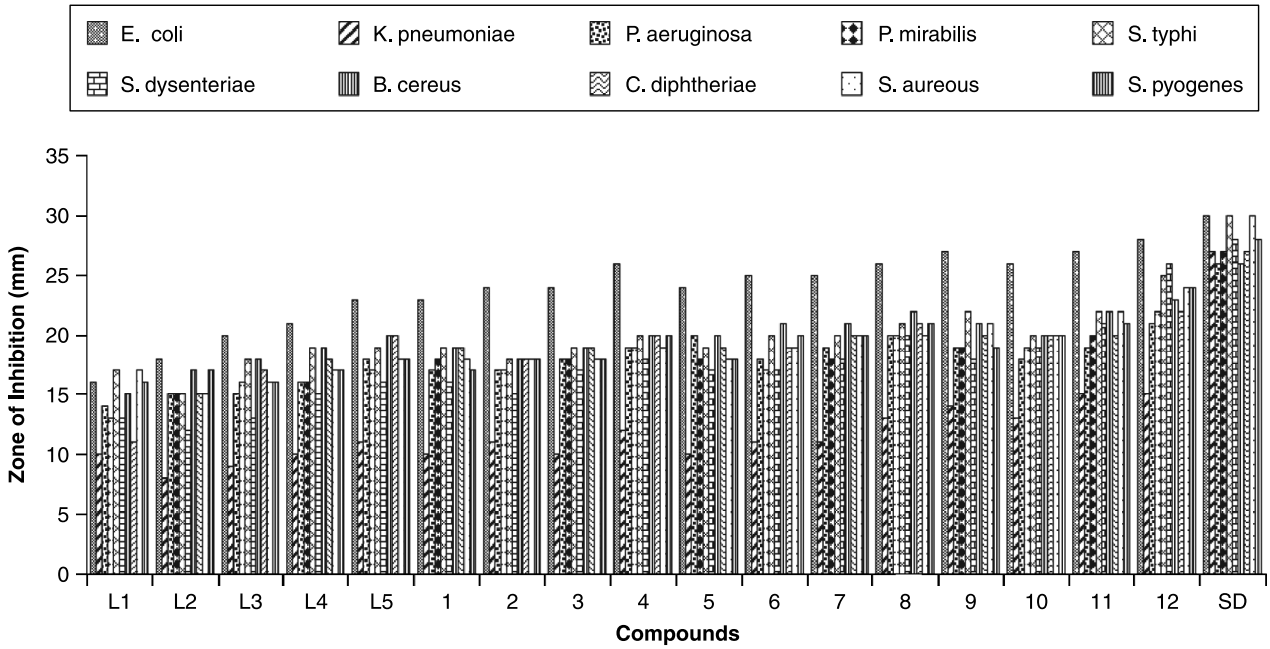


Figure 3. Comparison of Antibacterial Activity.

the other compounds of the series (**9**)–(**11**) where this chain was absent.

*Antifungal bioassay (*in-vitro*)*. The antifungal screening of all compounds was carried out against *T. longifusus, C. albican, A. flavus, M. canis, F. solani* and *C. glaberate* fungal strains according to the literature protocol [31]. The inhibition results were compared with those for the standard drugs miconazole and amphotericin B and individual synthesized compounds (Figure 4; Table III).

Minimum inhibitory concentration (MIC) for antibacterial activity. The preliminary antibacterial screening showed that compounds **(4),(6),(7),(8),(9),(10),(11)** and **(12)** were the most active ones (above 80%). These compounds were therefore, selected for minimum inhibitory concentration (MIC) studies (Table IV).

*Cytotoxic bioassay (*in-vitro*)*. All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al.* [33]. From the data recorded in Table V, it is evident that only two compounds, **(6)** and **(10)** displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive in this assay. Compound **(6)** showed activity

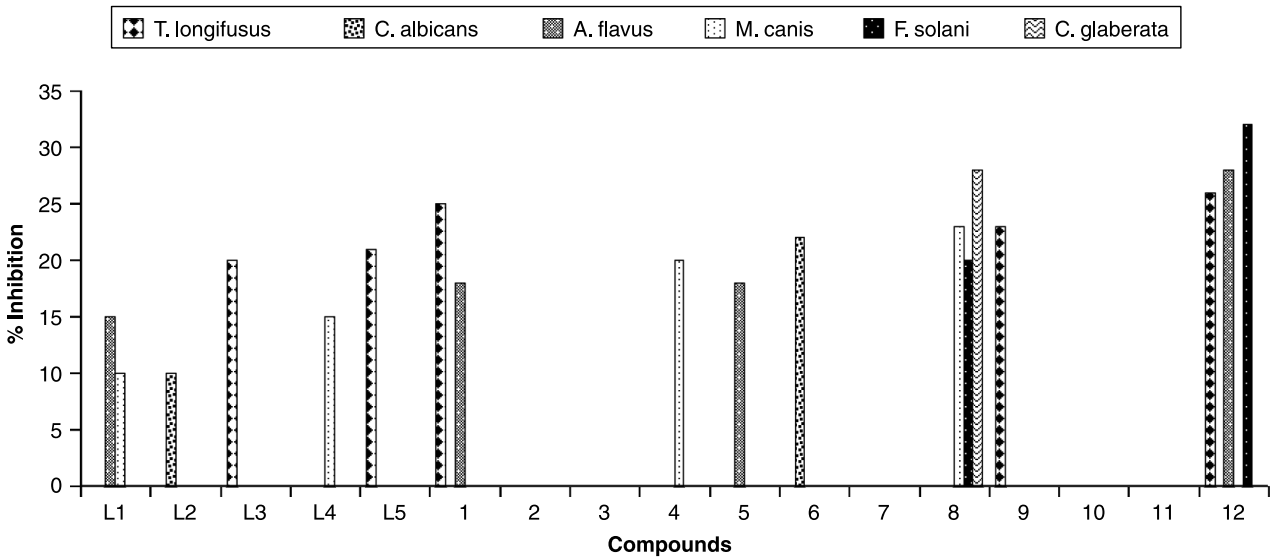


Figure 4. Comparison of Antifungal Activity.

Table III. Results of Antifungal Bioassay (concentration used 200 μg/mL).

	Compound																
Fungus	L_1	L_2	L_3	L_4	L_5	1	2	3	4	5	6	7	8	9	10	11	12	SD
(a)	00	00	20	00	21	25	00	00	00	00	00	00	00	23	00	00	26	A
(b)	00	10	00	00	00	00	00	00	00	00	22	00	00	00	00	00	00	B
(c)	15	00	00	00	00	18	00	00	00	18	00	00	00	00	00	00	28	C
(d)	10	00	00	15	00	00	00	00	20	00	00	00	23	00	00	00	00	D
(e)	00	00	00	00	00	00	00	00	00	00	00	00	20	00	00	00	32	E
(f)	00	00	00	00	00	00	00	00	00	00	00	00	28	00	00	00	00	F

(a) = T. longifusus, (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} moles/mL), B = Miconazole (110.8 μg/mL: 2.6626×10^{-7} moles/mL), C = Amphotericin B (20 μg/mL: 2.1642×10^{-8} moles/mL), D = Miconazole (98.4 μg/mL: 2.3647×10^{-7} moles/mL), E = Miconazole (73.25 μg/mL: 1.7603×10^{-7} moles/mL), F = Miconazole (110.8 μg/mL: 2.66266×10^{-7} moles/mL)

Table IV. Results of Minimum Inhibitory Concentration (MIC) of Selected Compounds (**4**), (**6**), (**7**), (**8**), (**9**), (**10**), (**11**), and (**12**) against Selected Bacteria.

No.	**12**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Gram-negative								
E. coli	3.834×10^{-8}	3.686×10^{-8}	7.425×10^{-8}	3.676×10^{-8}	1.425×10^{-7}	7.079×10^{-8}	3.564×10^{-8}	1.412×10^{-8}
P. aeruginosa	–	–	–	–	–	–	–	3.530×10^{-8}
P. mirabilis	–	–	–	–	–	–	–	3.530×10^{-8}
S. typhi	–	–	–	–	–	–	–	1.412×10^{-7}
S. dysenteriae	–	–	–	–	–	–	–	7.061×10^{-8}
Gram-positive								
B. cereus	–	7.372×10^{-8}	3.713×10^{-8}	1.470×10^{-7}	1.425×10^{-7}	–	3.564×10^{-8}	3.530×10^{-8}
C. diphtheriae	–	–	–	–	–	–	–	1.412×10^{-8}
S. pyogenes	–	–	–	–	–	–	–	1.412×10^{-7}

($LD_{50} = 4.644 \times 10^{-4}$ moles/mL) in the present series of compounds whereas the other active compound **(10)** of the series demonstrated activity, $LD_{50} = 4.106 \times 10^{-4}$ moles/mL. It was interesting to note that only copper complexes showed potent cytotoxicity whereas the other metal complexes did not. This activity relationship may help to serve as a basis for future direction towards the development of certain cytotoxic agents for clinical applications.

Table V. Brine Shrimp Bioassay Data of the Ligands (L_1)–(L_5) and their Metal (II) Complexes (**1**)–(**12**).

Compound	LD_{50} (Mm/ml)
L_1	$> 3.264 \times 10^{-3}$
L_2	$> 2.991 \times 10^{-3}$
L_3	$> 3.619 \times 10^{-3}$
L_4	$> 3.444 \times 10^{-3}$
L_5	$> 3.286 \times 10^{-3}$
1	$> 1.549 \times 10^{-3}$
2	$> 1.538 \times 10^{-3}$
3	$> 1.550 \times 10^{-3}$
4	$> 1.534 \times 10^{-3}$
5	$> 1.484 \times 10^{-3}$
6	4.644×10^{-4}
7	$> 1.485 \times 10^{-3}$
8	$> 1.470 \times 10^{-3}$
9	$> 1.425 \times 10^{-3}$
10	4.106×10^{-4}
11	$> 1.426 \times 10^{-3}$
12	$> 1.412 \times 10^{-3}$

The enhancement of antibacterial/antifungal activity in ligands **(L_3)–(L_5)** upon chelation may be rationalized on the basis of their structures. It has been suggested that chelation/coordination reduces the polarity of the metal ion [48–53] 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 [54–57] 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 into such compounds exhibit [57–61] extensive biological activities that may be responsible for increasing the 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.

Supplementary material

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 606492 for compound $C_{13}H_{12}N_2O_3S_1$. Copies of this information may be

Table VI. Selected Bond lengths [A] and Bond angles [deg] for ($\mathbf{L_3}$).

Bond distances							
S(1)–O(1)	1.436(2)	N(5)–H(64)	0.86(6)	C(15)–C(16)	1.384(6)	C(34)–C(35)	1.398(7)
S(1)–O(2)	1.438(3)	N(5)–H(62)	0.83(5)	C(15)–H(15A)	0.9500	C(34)–C(39)	1.401(7)
S(1)–N(1)	1.610(3)	N(6)–C(20)	1.276(6)	C(16)–C(17)	1.385(6)	C(35)–C(36)	1.375(7)
S(3)–O(10)	1.437(3)	C(1)–C(2)	1.384(6)	C(20)–H(20A)	0.9500	C(38)–H(38A)	0.9500
S(3)–N(5)	1.611(4)	C(2)–C(3)	1.386(6)	C(21)–C(22)	1.389(7)	C(40)–C(45)	1.386(6)
S(3)–C(27)	1.773(4)	C(2)–H(2C)	0.9500	C(21)–C(26)	1.409(7)	C(40)–C(41)	1.395(5)
S(4)–O(8)	1.435(3)	C(3)–C(4)	1.403(6)	C(22)–C(23)	1.381(7)	C(41)–C(42)	1.385(6)
S(4)–O(7)	1.436(3)	C(3)–H(3C)	0.9500	C(22)–H(22A)	0.9500	C(41)–H(41A)	0.9500
S(4)–N(7)	1.614(4)	C(4)–C(5)	1.390(6)	C(23)–C(24)	1.367(9)	C(42)–C(43)	1.380(6)
S(4)–C(40)	1.772(4)	C(5)–C(6)	1.381(6)	C(23)–H(23A)	0.9500	C(42)–H(42A)	0.9500
O(3)–C(13)	1.344(6)	C(5)–H(5D)	0.9500	C(24)–C(25)	1.385(8)	C(43)–C(44)	1.387(6)
O(3)–H(3)	0.8400	C(6)–H(6C)	0.9500	C(24)–H(24A)	0.9500	C(44)–C(45)	1.396(6)
O(12)–H(12)	0.8400	C(9)–H(9A)	0.9500	C(28)–H(28A)	0.9500	C(47)–C(52)	1.416(7)
N(3)–H(71)	0.75(6)	C(12)–H(12B)	0.9500	C(32)–H(32A)	0.9500	C(50)–H(50A)	0.9500
N(4)–C(33)	1.297(6)	C(14)–C(19)	1.376(5)	C(33)–C(34)	1.455(6)	C(51)–C(52)	1.402(6)
N(4)–C(30)	1.412(5)	C(14)–C(15)	1.386(6)	C(33)–H(33A)	0.9500	C(51)–H(51A)	0.9500
Bond Angles							
O(1)–S(1)–O(2)	117.78(17)	C(2)–C(3)–C(4)	119.6(4)	N(6)–C(20)–C(21)	120.6(4)		
O(1)–S(1)–N(1)	106.77(18)	C(2)–C(3)–H(3C)	120.2	N(6)–C(20)–H(20A)	119.7		
O(2)–S(1)–N(1)	108.01(17)	C(4)–C(3)–H(3C)	120.2	C(21)–C(20)–H(20A)	119.7		
O(1)–S(1)–C(1)	107.34(18)	C(5)–C(4)–C(3)	118.9(4)	C(22)–C(21)–C(26)	119.4(4)		
O(2)–S(1)–C(1)	107.98(17)	C(5)–C(4)–N(2)	117.2(4)	C(22)–C(21)–C(20)	119.6(5)		
O(4)–S(2)–C(14)	106.71(18)	C(1)–C(6)–C(5)	118.5(4)	C(24)–C(23)–C(22)	119.3(5)		
O(5)–S(2)–C(14)	108.37(17)	C(1)–C(6)–H(6C)	120.8	C(24)–C(23)–H(23A)	120.3		

Table VII. Crystal data for 4-[(2-hydroxybenzene)- amino]benzenesulfonamide ($\mathbf{L_3}$).

Identification code	shelxl
Formula weight	276.31
Temperature	173(2) K
Wavelength	0.71070 A
Crystal system, space group	Triclinic, P1
Unit cell dimensions	a = 8.9104(15) A alpha = 80.561(6) deg. b = 9.6705(15) A beta = 88.954(9) deg. c = 14.712(3) A gamma = 83.235(8) deg.
Volume	1241.9(4) A 3
Z, Calculated density	4, 1.478 Mg/m 3
Absorption coefficient	0.266 mm − 1
F(000)	576
Theta range for data collection	2.15 to 28.00 deg.
Limiting indices	−11 < = h < = 11, −12 < = k < = 12, −19 < = l < = 19
Reflections collected / unique	24746 / 11393 [R(int) = 0.0466]
Completeness to theta = 28.00	99.7%
Max. and min. transmission	1.0000 and 0.7845
Refinement method Full-matrix least-squares on F 2	
Data / restraints / parameters	11393 / 3 / 718
Goodness-of-fit on F 2	1.090
Final R indices [I > 2sigma(I)]	R1 = 0.0727, wR2 = 0.2028
R indices (all data)	R1 = 0.0824, wR2 = 0.2194
Absolute structure parameter	0.03(8)
Largest diff. peak and hole	1.265 and −0.526 e.Â

obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44-1223-336-033, E-mail: deposit@ccdc.cam.ac.uk or www:http://www.ccdc.cam.ac.uk)

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