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Metal based isatin-derived sulfonamides: Their synthesis, characterization, coordination behavior and biological activity

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Abstract

Some isatin derived sulfonamides and their transition metal [Co(II), Cu(II), Ni(II), Zn(II)] complexes have been synthesized and characterized. The structure of synthesized compounds and their nature of bonding have been inferred on the basis of their physical (magnetic susceptibility and conductivity measurements), analytical (elemental analyses) and spectral (IR, ^{1}H NMR and ^{13}C NMR) properties. An octahedral geometry has been suggested for Co(II), Ni(II) and Zn(II) and square-planar for Cu(II) complexes. In order to assess the antibacterial and antifungal behavior, the ligands and their metal(II) complexes were screened for their *in vitro* antibacterial activity against four Gram-negative species, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa and Salmonella typhi* and two Gram-positive species, *Staphylococcus aureus* and *Bacillus subtilis* and, for *in vitro* antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberata*. *In vitro* cytotoxic properties of all the compounds were also studied against *Artemia salina* by brine shrimp bioassay. The results of average antibacterial/antifungal activity showed that zinc(II) complexes were found to be the most active against one or more bacterial/fungal strains as compared to the other metal complexes.

Keywords: *Isatin, sulfonamides, complexes, antibacterial, antifungal, cytotoxicity*

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Introduction

Sulfonamides denote an important class of drugs [1]. Their significance appeared only when sulfanilamide was reported [2] to be the first metabolite of an antibacterial drug. Later on, a large number of sulfanilamide derivatives were synthesized, characterized and tested for antibacterial [3], antitumor [4], anti-carbonic anhydrase [5,6], diuretic [7,8], hypoglycemic [9], anti-thyroid [10] or protease inhibitory [11] activities. In the past decade, research has gained an increased interest in seeking new methodologies and targets that could work assertively in the treatment of bacteria and virus related ailments. Metal based therapies, so far, have come out to be the most effective approach for their treatment. Momentous part of which, is the ability of metal ions to bind with proteins and peptides. Simple as well as N-substituted sulfonamides have attracted much attention into this promising area of metal based therapy which was initially motivated by the successful introduction of metal complexes of sulfa drug (sulfadiazine) to prevent bacterial infections [12]. These metal complexes utilize themselves to slow release of the metal ions from the source utterly

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dependent on the binding nature. It is therefore, vital to be aware of the coordination behaviour, association and correlation of metals in biological systems.

In view of the versatile chemistry of both the sulfonamides and isatins, we have previously designed [13–15] a program not only to combine their chemistry but also to synthesize their respective metal based compounds in exploring their interesting structural and biological properties. In the same continuation, we wish to report in this paper some more isatin derived sulfonamides ($\mathbf{L_1}$)-($\mathbf{L_4}$) and their transition metal [(Co)(II), Cu(II), Ni(II), Zn(II)] complexes along with their binding behavior and *in vitro* antibacterial, antifungal and cytotoxic properties.

Experimental

Materials and measurements

All starting materials used were of chemical purity. The solvents used in the physical and spectral measurements were analytical grade. Metal (II) salts were used as chlorides. IR, ^{1}H NMR, ^{13}C NMR, UV-Visible spectra, CHN analyses, conductance and magnetic measurements were carried out using the respective instruments. *In vitro* antibacterial, antifungal and cytotoxic properties were studied at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Synthesis of ligands ($\boldsymbol{L_1}$)-($\boldsymbol{L_4}$)

General method. To a hot stirred solution of sulfonamide ($\mathbf{L_1}$) (5 mmole) in 1-butanol (35 mL) was added hot isatin (0.74 g, 5 mmole) solution in 1-butanol (20 mL). The mixture was refluxed for 12 h. The precipitates thus formed during refluxing, were cooled to room temperature and collected by suction filtration. Washing them thoroughly with ethanol (2 × 10 mL), afforded TLC pure product (71–77% yield).

N-(4,6-dimethylpyrimidin-2-yl)-4-{[2-oxo-1,2-dihydro-3H-indol-3-ylidene]-amino}benzenesulfonamide ($\boldsymbol{L_1}$). Yield 77% (1.57 g); m.p. = 260-261°C. IR (KBr, ν cm^{-1}): 3290 (NH), 3235 (NH), 1715 (C═O), 1585 (C═N), 1325, 1140 (S═O), 960 (S-N), 845 (C-S); ^{1}H NMR (DMSO-d$_6$, (ppm): 2.25 (s, 6H, 2CH$_3$), 6.74 (s, 1H, pyrimidine), 7.28-7.46 (m, 4H, indole), 7.75-7.81 (m, 4H, PhSO$_2$), 9.15 (s, 1H, azomethine), 10.24 (s, 1H, NH-indole), 10.29 (s, 1H, NHSO$_2$); ^{13}C NMR (DMSO-d$_6$, δ ppm): 25.1 (2CH$_3$-pyrimidine), 103.0 (C$_5$-pyrimidine), 122.6 (C$_3$,C$_5$-PhSO$_2$), 128.6 (C$_2$,C$_6$-PhSO$_2$), 138.2 (C$_1$-PhSO$_2$), 139.2-149.1 (C$_4$,C$_5$,C$_6$,C$_7$C$_8$,C$_9$-indole), 156.4 (C$_4$-PhSO$_2$), 160.0 (C═N, azomethine), 165.2 (C$_4$,C$_6$-pyrimidine), 168.5 (C$_2$-pyrimidine), 170.2 (C$_2$-indole); Anal. Calcd. for C$_{20}$H$_{17}$N$_5$O$_3$S (407.44): C, 58.96; H, 4.21; N, 17.19. Found: C, 58.93; H, 4.32; N, 17.18%.

N-(3,4-dimethylisoxazol-5-yl)-4-{[2-oxo-1,2-dihydro-3H-indol-3-ylidene]-amino}benzenesulfonamide ($\boldsymbol{L_2}$). Yield 71% (1.41 g); m.p. = 240-241°C. IR (KBr, ν cm^{-1}): 3290 (NH), 3235-3233 (NH), 1715 (C═O), 1585 (C═N), 1325, 1140 (S═O), 960 (S-N), 845 (C-S); ^{1}H NMR (DMSO-d$_6$, (ppm): 2.35 (m, 6H, CH$_3$), 7.28- 7.46 (m, 4H, indole), 7.75-7.81 (m, 4H, PhSO$_2$), 9.15 (s, 1H, azomethine), 10.25 (s, 1H, NH-indole),10.29 (s, 1H, NHSO$_2$),. ^{13}C NMR (DMSO-d$_6$, δ ppm): 9.5 (CH$_3$-isoxazol), 15.1 (CH$_3$-isoxazol), 100.5 (C$_4$-isoxazole), 122.6 (C$_3$,C$_5$-phSO$_2$), 128.6 (C$_2$,C$_6$-PhsSO$_2$), 138.2 (C$_1$-phSO$_2$), 139.2-149.1 (C$_4$,C$_5$,C$_6$,C$_7$C$_8$,C$_9$-indole), 156.4 (C$_4$-phSO$_2$), 158.9 (C$_5$-isoxazol), 159.9 (C$_3$-isoxazole), 160.0 (C═N, azomethine), 170.2 (C$_2$-indole); Anal. Calcd. for C$_{19}$H$_{16}$N$_4$O$_4$S (396.42): C, 57.57; H, 4.04; N, 14.13. Found: C, 57.66; H, 4.12; N, 14.08%.

N-(5-methylisoxazol-3-yl)-4-{[2-oxo-1,2-dihydro-3H-indol-3-ylidene] amino}benzenesulfonamide ($\boldsymbol{L_3}$). Yield 73% (1.40 g); m.p. = 229-230°C. IR (KBr, ν cm^{-1}): 3290 (NH), 3235 (NH), 1715 (C═O), 1585 (C═N), 1325, 1140 (S═O), 960 (S-N), 845 (C-S); ^{1}H NMR (DMSO-d$_6$, (ppm): 2.29 (s, 3H, CH3), 6.09 (s, 1H, isoxazol), 7.28-7.46 (m, 4H, indole), 7.75-7.81 (m, 4H, PhSO$_2$), 9.15 (s, 1H, azomethine), 10.25 (s, 1H, NH-indole), 10.30 (s, 1H, SO$_2$NH);. ^{13}C NMR (DMSO-d$_6$, δ ppm): 12.8 (CH$_3$-isoxazol), 95.0 (C$_4$-isoxazole), 122.6 (C$_3$,C$_5$-phSO$_2$), 128.6 (C$_2$,C$_6$-phSO$_2$), 138.2 (C$_1$-phSO$_2$), 139.2-149.1 (C$_4$,C$_5$,C$_6$,C$_7$C$_8$,C$_9$-indole); 150.0 (C$_3$-isoxazol), 156.4 (C$_4$-phSO$_2$), 159.6 (C$_5$-isoxazole), 160.0 (C═N, azomethine), 170.2 (C$_2$-indole); Anal. Calcd. for C$_{18}$H$_{14}$N$_4$O$_4$S (382.39): C, 56.54; H, 3.69; N, 14.65. Found: C, 56.50; H, 3.58; N, 14.52%.

N-(1,3-thiazol-2-yl)-4-{[2-oxo-1,2-dihydro-3H-indol-3-ylidene]amino}-benzenesulfonamide ($\boldsymbol{L_4}$). Yield 76% (1.46 g); m.p. = 255-256°C. IR (KBr, ν cm^{-1}): 3290 (NH), 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): 6.81-6.87 (m, 2H, thiazol), 9.15 (s, 1H, azomethine), 7.28-7.46 (m, 4H, indole), 7.75-7.81 (m, 4H, phSO$_2$), 10.27 (s, 1H, NH-indole). 10.32 (s, 1H, SO$_2$NH); ^{13}C NMR (DMSO-d$_6$, δ ppm): 108.0 (C$_4$-thiazol), 122.6 (C$_3$,C$_5$-phSO$_2$), 128.6 (C$_2$,C$_6$-phSO$_2$), 138.3 (C$_5$-thiazol), 139.1 (C$_1$-phSO$_2$), 139-149.1 (C$_4$,C$_5$,C$_6$,C$_7$C$_8$,C$_9$-indole); 156.4 (C$_4$-phSO$_2$), 160.0 (C═N, azomethine), 170.2 (C$_2$-indole), 171.7 (C$_2$-thiazol), Anal. Calcd. for C$_{16}$H$_{13}$N$_3$O$_4$S (384.43): C, 53.11; H, 3.15; N, 14.57. Found: C, 53.16; H, 3.26; N, 14.53%.

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Synthesis of metal (II) complexes

Synthesis of complex trans-*(Cl/Cl)-[Co(L_1)$_2$(Cl)$_2$] (**1**)*. To a hot magnetically stirred dioxane (10 mL) solution of (L_1) (0.81 g, 2 mmole), an aqueous solution (15 mL) of $CoCl_2.6H_2O$ (0.24 g, 1 mmole) was added. The mixture was refluxed for 3 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 × 5 mL) then with ether and dried. Recrystallization from 50% aqueous dioxane gave the desired product. Unfortunately only microcrystalline powders could be obtained, which could not to be used for X-ray structural determinations. The same method was used for the preparation of all other complexes (**2**)-(**16**).

Trans-*(Cl/Cl)-[Co(L_1)$_2$(Cl)$_2$] (**1**)*. 70% yield. M.p. 301-303°C. IR (KBr, ν cm^{-1}): 1570 (C═N), 1700 (C═O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 435 (M-N), 529 (M-O), 315 (M-Cl). Anal. Calcd. for $C_{40}H_{34}N_{10}O_6S_2Cl_2Co$ (944.74): C, 50.85; H, 3.63, N, 14.83. Found: C, 50.80; H, 3.53; N, 14.72%.

*Cu(L_1)$_2$]Cl$_2$] (**2**)*. 69% yield. M.p. = 310-312°C. IR (KBr, ν cm^{-1}): 1695 (C═O), 1565 (C═N), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 433 (M-N), 535 (M-O). Anal. Calcd. for $C_{40}H_{34}N_{10}O_6S_2Cl_2Cu$ (949.35): C, 50.61; H, 3.61; N,14.75; Found: C, 50.47; H, 3.67; N, 14.64.

Trans-*(Cl/Cl)-[Ni(L_1)$_2$(Cl)$_2$] (**3**)*. 67% yield. M.p. = 315-317°C. IR (KBr, ν cm^{-1}): 1560 (C═N), 1696(C═O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 430(M-N), 529(M-O), 315(M-Cl). Anal. Calcd. for $C_{40}H_{34}N_{10}O_6S_2Cl_2N$ (944.50): C, 50.87; H, 3.63; N, 14.83. Found: C, 50.89; H, 3.72; N, 14.80.

Trans-*(Cl/Cl)-Zn(L_1)$_2$(Cl)$_2$] (**4**)*. 71% yield. M.p. = 305-307°C. IR (KBr, ν cm^{-1}): 1568 (C═N), 1698 (C═O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 432 (M-N), 527 (M-O), 315 (M-Cl). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.28 (s, 6H, 2xCH_3), 6.78 (s, 1H, pyrimidine), 9.45 (s, 1H, azomethine), 7.32-7.53 (m, 4H, indole), 7.79-7.97 (m, 4H, phSO_2), 10.32 (s, 1H, NH-indole), 10.48 (s, 1H, SO_2NH); ^{13}C NMR (DMSO-d_6, δ ppm): 25.1 (CH_3-pyrimidine), 103.0 (C_5-pyrimidine), 122.6 (C_3,C_5-phenyl), 138.2 (C_1-phenyl), 128.6 (C_2,C_6-phenyl), 139.2-149.1 (C_4,C_5,C_6,$C_7$$C_8$,$C_9$-indole), 165.2 ($C_4$, C_6-pyrimidine), 166.3 (C_4-phSO_2), 168.5 (C_2-pyrimidine), 172.3 (C═N, azomethine), 179.5 (C_2-indole); Anal. Calcd. for $C_{40}H_{34}N_{10}O_6S_2Cl_2Zn$ (951.20): C, 50.51; H, 3.60; N, 14.72. Found: C, 50.55; H, 3.65; N, 14.53.

Trans-*(Cl/Cl)-[Co(L_2)$_2$(Cl)$_2$] (**5**)*. 74% yield. M.p. = 290-292°C. IR (KBr, ν cm^{-1}): 1565 (C═N), 1690 (C═O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 438 (M-N), 535 (M-O), 315 (M-Cl). Anal. Calcd. for $C_{38}H_{32}N_8O_8S_2Cl_2Co$ (922.69): C, 49.47; H, 3.50; N, 12.14. Found: C, 49.49; H, 3.45; N, 12.09.

*[Cu(L_2)$_2$]Cl$_2$ (**6**)*. 71% yield. M.p. = 299-301°C. IR (KBr, ν cm^{-1}): 1567 (C═N), 1699 (C═O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 439 (M-N), 520 (M-O). Anal. Calcd. for $C_{38}H_{32}N_8O_8S_2Cl_2Cu$ (927.30): C, 49.22; H, 3.48; N, 12.08. Found: C, 49.29; H, 3.44; N, 12.03%.

Trans-*(Cl/Cl)-[Ni(L_2)$_2$(Cl)$_2$] (**7**)*. 70% yield. M.p. = 284-286°C. IR (KBr, ν cm^{-1}): 1560 (C═N), 1692 (C═O), 1345, 1110 (SO_2), 960 (S-N), 845 (C-S), 433 (M-N), 530 (M-O), 315 (M-Cl). Anal. Calcd. for $C_{38}H_{32}N_8O_8S_2Cl_2Ni$ (922.44): C, 49.48; H, 3.50; N, 12.15. Found: C, 49.54; H, 3.43; N, 12.11%.

Trans-*(Cl/Cl)-[Zn(L_2)$_2$(Cl)$_2$] (**8**)*. 72% yield. m.p. = 294-296°C. IR (KBr, ν cm^{-1}): 1564 (C═N), 1694 (C═O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 430 (M-N), 534 (M-O), 315 (M-Cl). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.39 (m, 6H, CH_3), 9.45 (s, 1H, azomethine), 7.32-7.53 (m, 4H, indole), 7.79-7.97 (m, 4H, phSO_2), 10.32 (s, 1H, NH-indole).10.48 (s, 1H, SO_2NH), ^{13}C NMR (DMSO-d_6, δ ppm): 9.5 (CH_3-isoxazol), 15.1 (CH_3-isoxazol), 100.5 (C_4-isoxazole), 122.6 (C_3,C_5-phSO_2), 128.6 (C_2,C_6-phSO_2), 138.2 (C_1-phSO_2), 139.2-149.1 (C_4,C_5,C_6,$C_7$$C_8$,$C_9$-indole), 158.9 ($C_5$-isoxazol), 159.8 ($C_3$-isoxazole), 165.2 ($C_4$-ph$SO_2$), 172.3 (C═N, azomethine), 179.5 (C_2-indole); Anal. Calcd. for $C_{38}H_{32}N_8O_8S_2Cl_2$ Zn (929.14): C, 49.12; H, 3.47; N, 12.06. Found: C, 49.19; H, 3.41; N, 12.00%.

Trans-*(Cl/Cl)-[Co(L_3)$_2$(Cl)$_2$] (**9**)*. 68% yield. M.p. = 311-313°C. IR (KBr, ν cm^{-1}): 1565 (C═N), 1696 (C═O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 434 (M-N), 535 (M-O), 315 (M-Cl). Anal. Calcd. for $C_{36}H_{28}N_8O_8S_2Cl_2Co$ (894.63): C, 48.33; H, 3.15; N, 12.52. Found: C, 48.28; H, 3.20; N, 12.48%.

*[Cu(L_3)$_2$]Cl$_2$ (**10**)*. 72% yield. M.p. = 304-306°C. IR (KBr, ν cm^{-1}): 1563 (C═N), 1693 (C═O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 435 (M-N), 522 (M-O). Anal. Calcd. for $C_{36}H_{28}N_8O_8S_2Cl_2Cu$ (899.25): C, 48.08; H, 3.14; N, 12.46. Found: C, 48.18; H, 3.16; N, 12.41%.

Trans-*(Cl/Cl)-[Ni(L_3)$_2$(Cl)$_2$] (**11**)*. 76% yield. M.p. = 308-310°C. IR (KBr, ν cm^{-1}): 1562 (C═N), 1698(C═O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 430(M-N), 525(M-O), 315(M-Cl). Anal. Calcd. for $C_{36}H_{28}N_8O_8S_2Cl_2Ni$ (894.39): C, 48.35; H, 3.16; N, 12.53. Found: C, 48.29; H, 3.10; N, 12.433%.

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Trans-*(Cl/Cl)-[Zn(L$_3$)$_2$(Cl)$_2$]* (**12**). 70% yield. m.p. = 295-297°C. IR (KBr, ν cm^{-1}): 1561 (C=N), 1691 (C=O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 440 (M-N), 520 (M-O), 315 (M-Cl). ^{1}H NMR (DMSO-d$_6$, δ ppm): 2.34 (s, 3H, CH_3), 6.13 (s, 1H, isoxazol), 9.45 (s, 1H, azomethine), 7.32- 7.53 (m, 4H, indole), 7.79-7.97 (m, 4H, phSO_2), 10.32 (s, 1H, NH-indole), 10.47 (s, 1H, SO_2NH), ^{13}C NMR (DMSO-d$_6$, δ ppm): 12.8 (CH_3-isoxazol), 95.0 (C_4-isoxazole), 122.6 (C_3,C_5-phSO_2), 128.6 (C_2,C_6-phSO_2), 138.2 (C_1-phSO_2), 139.2-149.1 (C_4,C_5,C_6,$C_7$$C_8$,$C_9$-indole), 150.0 ($C_3$-isoxazol), 159.6 ($C_5$-isoxazole), 165.2 ($C_4$-ph$SO_2$), 172.3 (C=N, azomethine), 179.5 (C_2-indole); Anal. Calcd. for 901.09 ($C_{36}H_{28}N_8O_8S_2Cl_2$ Zn): C, 47.99; H, 3.13; N, 12.43. Found: C, 47.87; H, 3.18; N, 12.40%.

Trans-*(Cl/Cl)-[Co(L$_4$)$_2$(Cl)$_2$]* (**13**). 72% yield. M.p. = 282-284°C. (KBr, ν cm^{-1}): 1560 (C=N), 1690 (C=O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 425 (M-N), 526 (M-O), 315 (M-Cl). Anal. Calcd. for $C_{34}H_{24}N_8O_6S_4Cl_2Co$ (898.71): C, 45.44; H, 2.69; N, 12.47. Found: C, 45.51; H, 2.75; N, 12.42%.

[Cu(L$_4$)$_2$]Cl$_2$ (**14**). 69% yield. M.p. = 287-299°C. IR (KBr, ν cm^{-1}): 1563 (C=N), 1693 (C=O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 433 (M-N), 533 (M-O). Anal. Calcd. for $C_{34}H_{24}N_8O_6S_4Cl_2Cu$ (903.33): C, 45.21; H, 2.68; N, 12.40. Found: C, 45.28; H, 2.77; N, 12.35%.

Trans-*(Cl/Cl)-[Ni(L$_4$)$_2$(Cl)$_2$]* (**15**). 75% yield. M.p. = 278-280°C. IR (KBr, ν cm^{-1}): 1560 (C=N), 1695 (C=O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 435 (M-N), 535 (M-O), 315 (M-Cl). Anal. Calcd. for $C_{34}H_{24}N_8O_6S_4Cl_2Ni$ (898.47): C, 45.45; H, 2.69; N, 12.47. Found: C, 45.53; H, 2.73; N, 12.33%.

Trans-*(Cl/Cl)-[Zn(L$_4$)$_2$(Cl)$_2$]* (**16**). 72% yield. M.p. = 293-295°C. IR (KBr, ν cm^{-1}): 1565 (C=N), 1698 (C=O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 431 (M-N), 528 (M-O), 315 (M-Cl). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 6.85-6.95 (m, 2H, thiazol), 9.45 (s, 1H, azomethine), 7.32- 7.53 (m, 4H, indole), 7.79-7.97 (m, 4H, ph-SO_2), 10.32 (s, 1H, NH-indole), 10.60 (s, 1H, SO_2NH), ^{13}C NMR (DMSO-d$_6$, δ ppm): 108.0 (C_4-thiazol), 128.6 (C_2,C_6-phSO_2), 122.6 (C_3,C_5-phSO_2), 138.3 (C_5-thiazol), 139.1 (C_1-phSO_2), 139.5-149.1 (C_4,C_5,C_6, $C_7$$C_8$,$C_9$-indole), 165.2 ($C_4$-ph$SO_2$), 171.7 ($C_2$-thiazol), 172.3 (C=N, azomethine), 179.5 (C_2-indole); Anal. Calcd. for 905.17 ($C_{34}H_{24}N_8O_6S_4$ Cl_2 Zn): C, 45.12; H, 2.67; N, 12.38. Found: C, 45.21; H, 2.69; N, 12.37%.

Biological activity

*Antibacterial bioassay (*in vitro*)*. All the synthesized compounds (**L$_1$**)-(**L$_4$**) and metal(II) complexes (**1**)-(**16**) were screened *in vitro* for their antibacterial activity against four Gram-negative (*Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa and Salmonella typhi*) and two Gram-positive species (*Staphylococcus aureus* and *Bacillus subtilis*) bacterial strains by the agar-well diffusion method [16,17]. The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Two to eight hours old bacterial inocula containing approximately 10^4-10^6 colony-forming units (CFU/mL) were spread on the surface of the nutrient agar using the help of a sterile cotton swab. The recommended concentration [18] 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 (*Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida 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 (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 as % of inhibition and compared [16] with standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration (*MIC*). Compounds containing high antibacterial activity (over 80%) were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion technique by preparing discs containing 10, 25, 50 and 100 μg/mL of the compounds and applying the protocol [19,20].

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

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darkened while the matter compartment was opened to ordinary light. After two days 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 sea water and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h the number of survivors was counted. Data were analyzed by Finney computer program to determine the LD_{50} values [21].

Results and discussion

Chemistry

Ligands. The sulfonamide derived ligands ($\mathbf{L_1}$)-($\mathbf{L_4}$) were prepared as shown in Scheme 1. All ligands were only soluble in dioxane, DMF and DMSO. The composition of the ligands is consistent with their spectroscopic ^1H, ^{13}C NMR and microanalytical data.

Metal (II) complexes. The metal(II) complexes (**1**)-(**16**) of the ligands ($\mathbf{L_1}$)-($\mathbf{L_4}$) were prepared (Figure 5) according to the following equations:

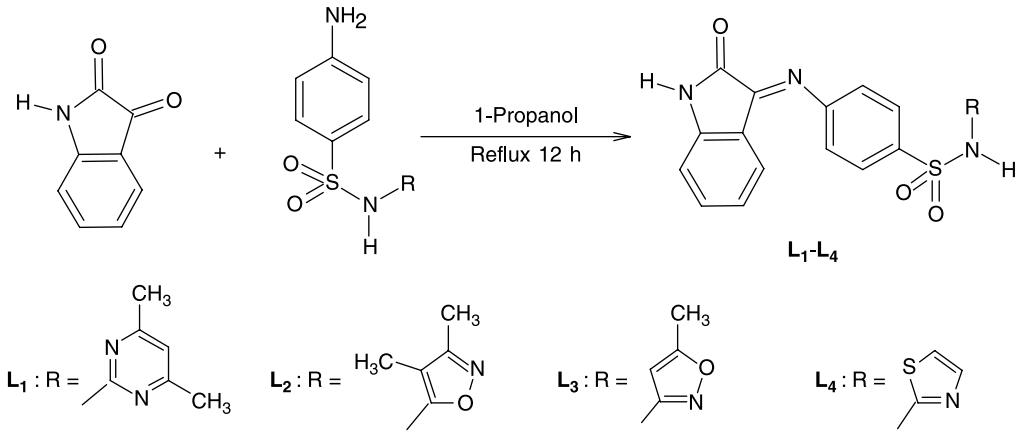
$$MCl_2nH_2O + 2\,\text{Ligand(Li)} \rightarrow [M(Li)_2(Cl)_2]$$
$$\text{or } [Cu(Li)_2](Cl)_2$$
$$M = \text{Co(II), Ni(II) \& Zn(II)}. \quad Li = (\mathbf{L_1})\text{-}(\mathbf{L_4}).$$

Conductance and magnetic measurements. The molar conductance values (in DMF) for cobalt, nickel and zinc complexes fall within the range 11-16 $\Omega^{-1}\,cm^2\,mol^{-1}$, showing their non-electrolytic [22] nature. However, the copper(II) complexes have molar conductance values in the range 86-89 $\Omega^{-1}\,cm^2\,mol^{-1}$ suggesting their electrolytic behavior. This in turn, proposes that the chloride ions are coordinated with the cobalt(II), nickel(II) and zinc(II) metal ions but not coordinated with the copper(II) complexes and staying outside the coordination sphere as anions. The room temperature magnetic moment values of the complexes are given in Table I.

The magnetic susceptibility measurements (4.1-4.4 B.M) for the solid Co (II) complexes are indicative of three unpaired electrons per Co (II) ion suggesting consistency with their octahedral environment. The magnetic moment values (1.37-1.53 B.M.) measured for the copper(II) complexes lie in the range expected to contain one unpaired electron for square-planar geometry [23]. The measured values (3.20-3.28 B.M.) for the nickel(II) complexes suggest [24] 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 its metal complexes are given in experimental and in Table I. All ligands contain various potential electron pair donor sites. In the IR spectra of the ligands, sharp bands observed at 1585 and 1715 cm^{-1} are assigned [48] to the ν(C = N) and ν(C = O) modes. Evidences 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} (1560-1570 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 520-535 cm^{-1} due to the ν(M-N) and ν (M-O) bands in all the complexes [25,26].

The bands in the ligand due to $\nu_{asymm}(SO_2)$ and $\nu_{symm}(SO_2)$ appear at 1325 and 1140 cm^{-1}, respectively. These bands remain unchanged in the complexes, indicating that this group is not participating



Scheme 1. Preparation of ligands ($\mathbf{L_1}$)-($\mathbf{L_4}$).

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Table I. Analytical conductivity, magnetic and spectral data of metal (II) complexes.

No.	Ω_M (Ω^{-1} cm^2 mol^{-1})	B.M (μ_{eff})	λ_{max} (cm^{-1})	IR (cm^{-1})
1.	14.2	4.87	7355, 17365, 20375, 29210	1570(C=N), 1700(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 435(M-N), 529(M-O), 315(M-Cl)
2.	86.0	1.41	14825, 19250, 30240	1565(C=N), 1695(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 433(M-N), 535(M-O)
3.	11.0	3.23	10395, 15835, 26395, 30105	1560(C=N), 1696(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 430(M-N), 529(M-O), 315(M-Cl)
4.	15.5	Dia	28955	1568(C=N), 1698(C=O), 1325, 1140(SO_2), 960 (S-N), 845 (C-S), 432(M-N), 527(M-O), 315(M-Cl)
5.	16.0	4.90	7210, 17285, 20525, 29225	1565(C=N), 1690(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 438(M-N), 535(M-O), 315(M-Cl)
6.	89.0	1.44	15210, 19235, 30155	1567(C=N), 1699(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 439(M-N), 520(M-O)
7.	15.3	3.28	10440, 15825, 26425, 30235	1560(C=N), 1692(C=O), 1345, 1110(SO_2), 960 (S-N), 845(C-S), 433(M-N), 530(M-O), 315(M-Cl)
8.	14.7	Dia	29125	1564(C=N), 1694(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 430(M-N), 534(M-O), 315(M-Cl)
9.	11.9	4.88	7325, 17390, 20505, 29245	1565(C=N), 1696(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 434(M-N), 535(M-O), 315(M-Cl)
10.	87.0	1.53	14985, 19325, 30225	1563(C=N), 1693 (C=O), 1325, 1140 (SO_2), 960 (S-N), 845 (C-S), 435(M-N), 522 (M-O)
11.	14.8	3.24	10445, 15690, 26510, 30230	1562 (C=N), 1698(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 430(M-N), 525(M-O), 315(M-Cl)
12.	15.9	Dia	28880	1561(C=N), 1691(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 440(M-N), 520(M-O), 315(M-Cl)
13.	16.0	4.90	7305, 17295, 20465, 29195	1560(C=N), 1690(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 425(M-N), 526(M-O), 315(M-Cl)
14.	87.2	1.37	15220, 19300, 30185	1563(C=N), 1693(C=O), 1325, 1140(SO_2), 960 (S-N), 845 (C-S), 433(M-N), 533 (M-O)
15.	15.8	3.20	10380, 15740, 26355, 30155	1560(C=N), 1695(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 435(M-N), 535(M-O), 315(M-Cl)
16.	14.6	Dia	28765	1565(C=N), 1698(C=O), 1325, 1140(SO_2), 960 (S-N), 845(C-S), 431(M-N), 528(M-O), 315(M-Cl)

in coordination. This is supported by the unchanged ν(S-N) and ν(C-S) modes appearing at 960 and 845 cm^{-1}, respectively [27], in the ligands after complexation. All the other potential electron pair donor sites of the ligands do not participate in coordination as their IR frequencies remain unchanged after complexation. A new band appearing at 315 cm^{-1} assigned [28] 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 [29] that the chloride atoms are not coordinated with the copper metal ions.

^{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 spectral data along with the possible assignments is recorded in the experimental part. All the protons due to heteroaromatic/aromatic groups were found as to be in their expected region [30]. The conclusions drawn from these studies lend further support to the mode of bonding discussed in their IR spectra. Also, the isatin protons underwent downfield shift due to the increased conjugation [31] 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.

^{13}C NMR spectra. ^{13}C NMR spectra of the free ligands and their diamagnetic zinc(II) complexes were also recorded in DMSO-d_6. The ^{13}C NMR spectral data along with the possible assignments is recorded in the experimental part. The carbons atoms due to heteroaromatic/aromatic groups were found as to be in their expected region. The conclusions drawn from these studies present further support to the mode of bonding discussed in their IR and ^{1}H NMR spectra. Downfield shifting of the —CH=N- signal from 160.0 ppm in the ligand to 172.3 ppm in its metal (II) complexes revealed coordination of the azomethine nitrogen to the metal atom [30]. All other carbons near coordination sites underwent downfield shifting by 3.5-8.5 ppm due to the increased conjugation and coordination with the metal atoms. Furthermore, the presence of the number of carbons agrees well with the expected values [31].

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Electronic spectra. The Co(II) complexes exhibited well-resolved, low-energy bands at 7,210-7,355 cm^{-1}, 17,285-17,390 cm^{-1} and a strong high-energy band at 20,375-20,525 cm^{-1} (Table I) which are assigned [32] to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ for a high-spin octahedral geometry. A high intensity band at 29,195-29,245 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 [33] consistency with their octahedral environment (Figure 1A).

The electronic spectra of the Cu(II) complexes (Table I) showed two low-energy weak bands at 14,825-15,220 cm^{-1} and 19,235-19,325 cm^{-1} and a strong high-energy band at 30,155-30,240 cm^{-1} and may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively. The strong high-energy band, in turn, is assigned to metal → ligand charge transfer. Also, the magnetic moment values for the Cu(II) are indicative their square-planar geometry [32] (Figure 5B).

The electronic spectra of the Ni(II) complexes showed d-d bands in the region 10,380-10,445, 15,690-15,825 and 26,355-26,510 cm^{-1}. These are assigned 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 30,105-30,235 cm^{-1} was assigned to (metal → ligand) charge transfer. The magnetic measurements showed two unpaired electrons per Ni(II) ion suggesting [34] also an octahedral geometry for the Ni(II) complexes (Figure 2A). The electronic spectra of the Zn(II) complexes exhibited only a high-intensity band at 28,765-29,125 cm^{-1} and are assigned to a ligand-metal charge transfer.

Biological activity

*Antibacterial bioassay (*in vitro*)*. All compounds were tested against four Gram-negative (*E. coli*, *S. flexenari*, *P. aeruginosa*, *S. typhi*) and two Gram-positive (*S. aureus*, *B. subtilis*) bacterial strains (Table II) according to literature protocol [16,18]. The results were compared with those of the standard drug imipenum (Figure 2). All ligands and their metal(II) complexes showed moderate to significant activity against all Gram-negative and Gram-positive bacterial strains except the activity of all compounds against strain *(c)* where no moderate to significant activity was observed. Compounds (**1**)-(**16**) exhibited overall

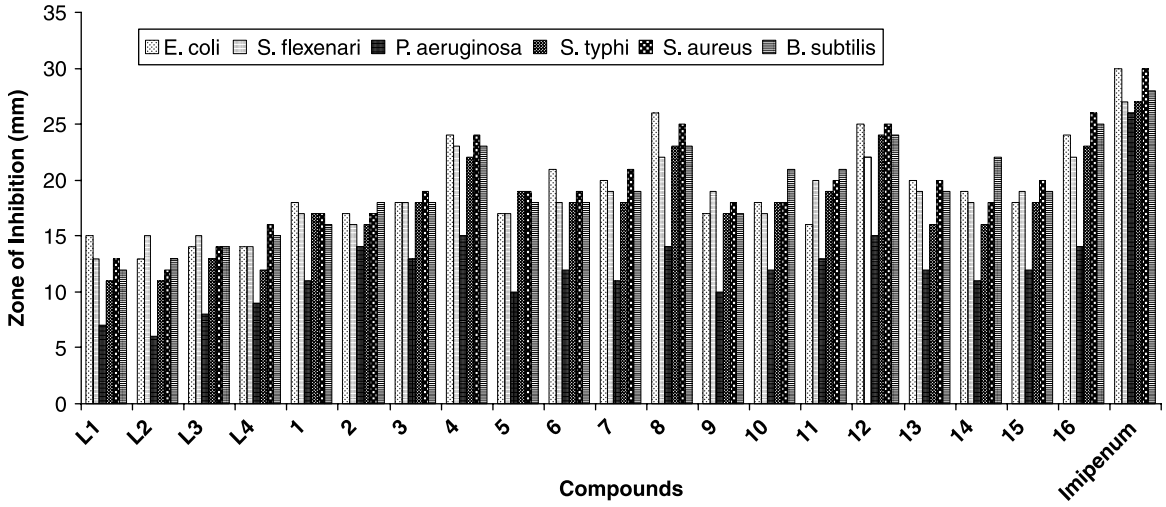

Figure 1. Comparison of antibacterial activity.

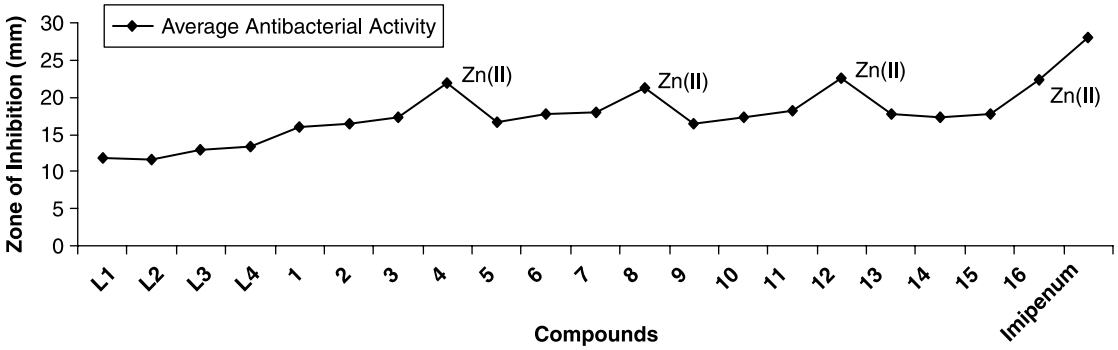

Figure 2. Average antibacterial activity of ligands versus metal (II) complexes.

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Table II. Antibacterial bioassay (concentration used 1 mg/mL of DMSO) of ligands and metal (II) complexes.

	Bacterial Strains					
	Gram-negative				Gram-positive	
Compd.	*E. colii* ***(a)***	*S..flexeneri* ***(b)***	*P. aeruginosa* ***(c)***	*S. typhi* ***(d)***	*S. aureous* ***(e)***	*B. subtilis* ***(f)***
L_1	15	13	7	11	13	12
L_2	13	15	6	11	12	13
L_3	14	15	8	13	14	14
L_4	14	14	9	12	16	15
1	18	17	11	17	17	16
2	17	16	14	16	17	18
3	18	18	13	18	19	18
4	24	23	15	22	24	23
5	17	17	10	19	19	18
6	21	18	12	18	19	18
7	20	19	11	18	21	19
8	26	22	14	23	25	23
9	17	19	10	17	18	17
10	18	17	12	18	18	21
11	16	20	13	19	20	21
12	25	22	15	24	20	21
13	20	19	12	16	20	19
14	19	18	11	16	18	22
15	18	19	12	18	20	19
16	24	22	14	23	26	25
SD	30	27	26	27	30	28
M1	5	4	5	4	4	5
M2	4	4	4	5	5	4
M3	4	3	4	4	4	4
M4	5	4	5	5	5	5

(a) = *E. coli* ***(b)*** = *S. flexenari* ***(c)*** = *P. aeruginosa* ***(d)*** = *S. typhi* ***(e)*** = *S. aureus* ***(f)*** = *B. subtilis* <10:weak; >10:moderate; > 16: Significant SD = Standard Drug (Imipenum), M_1 = $CoCl_2.6H_2O$, M_2 = $CuCl_2$, M_3 = $NiCl_2$, M_4 = $ZnCl_2$

a significant activity against *E. coli*, *S. flexenari*, *S. typhi*, *S. aureus and B. subtilis*. However, a moderate activity was observed by ligands (**L_1**)-(**L_4**) against all bacterial strains except *(c)* where they showed weak activity. Antibacterial activity is overall enhanced after complexation of the ligands. However the Zinc (II) complexes of all the ligands were observed to be the most active against all species (Figure 3). However, cobalt(II), copper(II) and nickel(II) metal complexes overall, showed moderate-good activity.

In another experiment, simple metal salts as chlorides were tested against the same bacterial species in order to evaluate an individual role of the corresponding metal ions in antibacterial activity. It was found that all metal

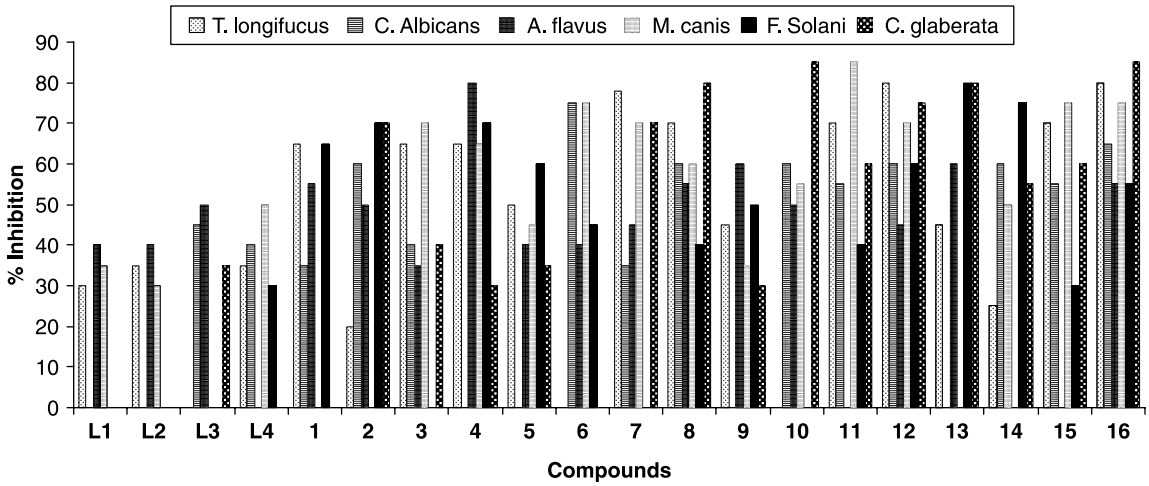

Figure 3. Comparison of antifungal activity.

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ions showed weak activity (5–7 mm) however, the zinc salt showed slightly more activity (8–9 mm) than cobalt, copper and nickel salts but in the range as weak activity.

*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 (Table III) according to the literature protocol [16]. All synthesized compounds showed good antifungal activity against different fungal strains. Compound (**8**), (**12**) and (**16**) showed good antifungal activity against all the fungal strains. The results of inhibition were compared with the results of inhibition of standard drugs miconazole and amphotericin B (Table III) and individual synthesized compounds were also compared (Figure 4). Effect of metal complexation on antifungal activity of the ligands can be seen in Figure 5.

Minimum inhibitory concentration (MIC) for antibacterial activity. The preliminary antibacterial screening showed that compounds (**4**), (**8**), (**12**) and (**16**) were the most active ones (above 80%). These compounds were therefore, selected for antibacterial 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. [20]. From the data recorded in Table V,

Table III. Antifungal bioassay (concentration used 200 μg/mL) of ligands and metal (II) complexes.

	Fungal Strains					
Compd.	*T. longifucus* ***(a)***	*C. albicans* ***(b)***	*A. flavus* ***(c)***	*M. canis* ***(d)***	*F. solani* ***(e)***	*C. glaberata* ***(f)***
$\mathbf{L_1}$	30	0	40	35	0	0
$\mathbf{L_2}$	35	0	40	30	0	0
$\mathbf{L_3}$	0	45	50	0	0	35
$\mathbf{L_4}$	35	40	0	50	30	0
1	65	35	55	0	65	0
2	20	60	50	0	70	70
3	65	40	35	70	0	40
4	65	0	80	65	70	30
5	50	0	40	45	60	35
6	0	75	40	75	45	0
7	78	35	45	70	0	70
8	70	60	55	60	40	80
9	45	0	60	35	50	30
10	0	60	50	55	0	85
11	70	55	0	85	40	60
12	80	60	45	70	60	75
13	45	0	60	0	80	80
14	25	60	50	0	75	55
15	70	55	0	75	30	60
16	80	65	55	75	55	85
SD	A	B	C	D	E	F

SD = Standard Drugs MIC μg/mL; A = Miconazole (70 μg/mL:1.6822 × 10^{-7} M/mL),B = Miconazole (110.8 μg/mL:2.6626 × 10^{-7} M/mL), C = Amphotericin B (20 μg/mL:2.1642 × 10^{-8} M/mL), D = Miconazole (98.4 μg/mL:2.3647 × 10^{-7} M/mL), E = Miconazole (73.25 μg/mL: 1.7603 × 10^{-7} M/mL), F = Miconazole (110.8 μg/mL: 2.66266 × 10^{-7} M/mL).

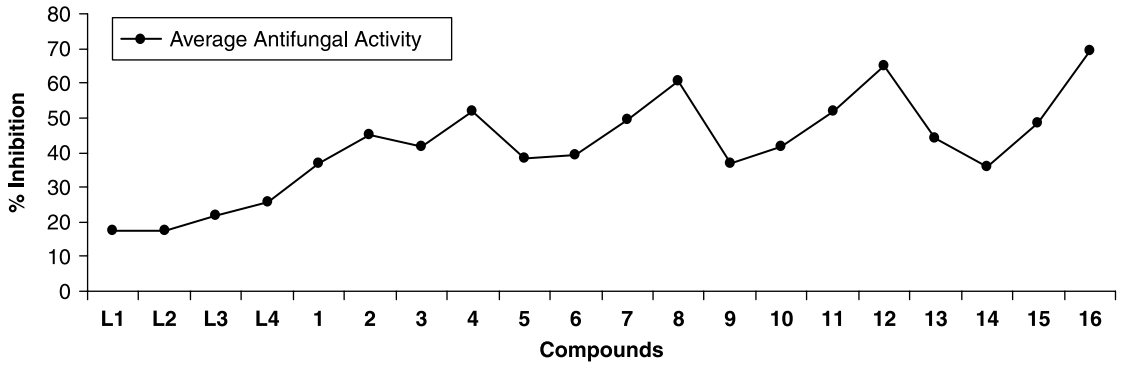

Figure 4. Average antifungal activity in ligands versus metal (II) complexes.

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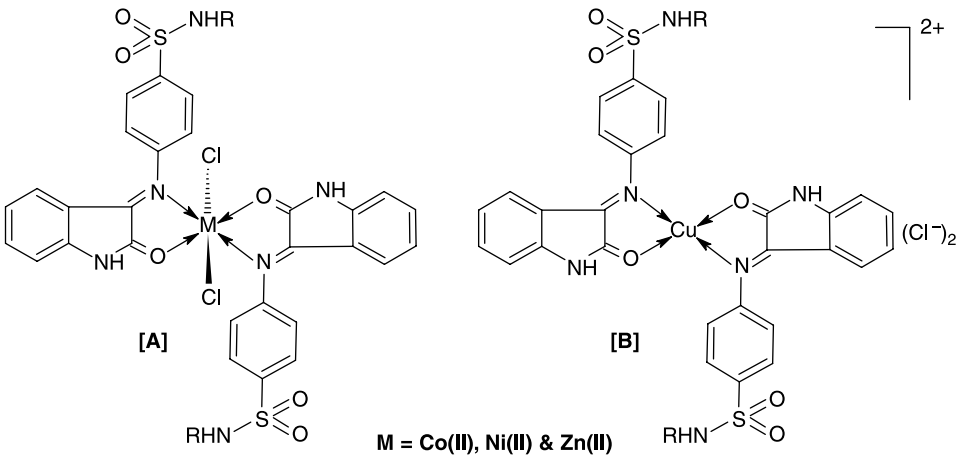

Figure 5. Proposed structures of the metal (II) complexes.

Table IV. Minimum inhibitory concentration (μg/mL) of the selected compounds (**4**), (**8**), (**12**) and (**16**) against selected bacteria.

	Gram-negative			Gram-positive	
Compd.	*E. coli*	*S. flexenari*	*S. typhi*	*S. aureus*	*B. subtilis*
4	1.051×10^{-7}	5.256×10^{-8}	1.051×10^{-7}	5.256×10^{-8}	2.628×10^{-8}
8	5.381×10^{-8}	2.691×10^{-8}	5.381×10^{-8}	1.076×10^{-7}	1.076×10^{-8}
12	5.549×10^{-8}	1.110×10^{-7}	2.750×10^{-8}	5.549×10^{-8}	1.110×10^{-8}
16	1.105×10^{-7}	5.524×10^{-8}	2.762×10^{-8}	5.524×10^{-8}	1.105×10^{-7}

it is evident that two compounds, (**2**), (**6**), (**10**) and (**14**) displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive for this assay. The compound (**2**) showed activity ($LD_{50} = 4.529 \times 10^{-4}$ μg/mL), compound (**6**) showed activity ($LD_{50} = 5.683 \times 10^{-4}$ μg/mL), compound (**10**) showed activity ($LD_{50} = 6.372 \times 10^{-4}$ μg/mL), compound (**14**) showed activity ($LD_{50} = 5.513 \times 10^{-4}$ μg/mL) in the present series of compounds. It was interesting to note that only copper complexes showed potent cytotoxicity whereas the other metal complexes did not. This activity relationship may help to serve as a basis for future direction towards the development of certain cytotoxic agents for clinical applications.

Table V. Brine shrimp bioassay data of the ligands (L_1)-(L_4) and their metal (II) complexes (**1**)-(**16**).

Compound	LD_{50} (μg/mL)	Compound	LD_{50} (μg/mL)
L_1	$> 2.454 \times 10^{-3}$	**7**	$> 1.084 \times 10^{-3}$
L_2	$> 2.523 \times 10^{-3}$	**8**	$> 1.076 \times 10^{-3}$
L_3	$> 2.615 \times 10^{-3}$	**9**	$> 1.118 \times 10^{-3}$
L_4	$> 2.601 \times 10^{-3}$	**10**	6.372×10^{-4}
1	$> 1.058 \times 10^{-3}$	**11**	$> 1.118 \times 10^{-3}$
2	4.529×10^{-4}	**12**	$> 1.110 \times 10^{-3}$
3	$> 1.059 \times 10^{-3}$	**13**	$> 1.113 \times 10^{-3}$
4	$> 1.051 \times 10^{-3}$	**14**	5.513×10^{-4}
5	$> 1.084 \times 10^{-3}$	**15**	$> 1.113 \times 10^{-3}$
6	5.683×10^{-4}	**16**	$> 1.105 \times 10^{-3}$

Conclusion

The enhancement of antibacterial/antifungal activity in ligands upon chelation/coordination is rationalized on the basis of their structures and the mode of coordination with the metals. It has been suggested that chelation reduces the polarity of the metal ion [35–37] on partial sharing of its positive charge with the donor groups. The process of chelation thus increases the lipophilic nature of the metal atom which, in turn, favors [38,39] its permeation through the lipoid layer of cell membrane of the micro-organism. It has also been suggested that some functional groups such as azomethine or hetero-aromatics present in these compounds display [40,41] extensive biological activities.

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