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In vitro antibacterial, antifungal & cytotoxic activity of some isonicotinoylhydrazide Schiff's bases and their cobalt (II), copper (II), nickel (II) and zinc (II) complexes

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Abstract

Isonicotinoylhydrazide Schiff's bases formed by the reaction of substituted and unsubstituted furyl-2-carboxaldehyde and thiophene-2-carboxaldehyde with isoniazid and, their Co (II), Cu (II), Ni (II) and Zn (II) complexes have been synthesized, characterized and screened for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Bacillus cereus*, *Corynebacterium diphtheriae*, *Staphylococcus aureus* and *Streptococcus pyogenes* bacterial strains and for *in vitro* antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata*. The results of these studies show the metal complexes to be more antibacterial and antifungal against one or more bacterial/fungal strains as compared to the uncomplexed compounds. The brine shrimp bioassay indicated Schiff's bases, L^3 and L^6 and, their Cu (II) and Ni (II) metal complexes to be cytotoxic against *Artemia salina*, while all other compounds were inactive ($LD_{50} > 1000$).

Keywords: *Isonicotinoyl, Schiff's Bases, metal complexes, antibacterial, antifungal, cytotoxicity*

Introduction

Tuberculosis continues to be a devastating disease worldwide. The increasing incidence of drug-resistance is emerging throughout as a major problem in drug therapy [1]. The related mucormycosis is also a life-threatening infection with a poor prognosis unless diagnosed in time. An aggressive antibacterial/antifungal/antiviral therapy combined with surgical debridement is in practice but no remarkable success in eliminating the actual cause has been fully achieved. Filamentous fungi of the order *Mucorales* cause this mycosis. Neutropenic hosts with debilitating conditions such as diabetic ketoacidosis, protein-calorie malnutrition and AIDS are preferentially predisposed to the infection [2–4]. Unfortunately, the majority of etiologic agents, including *Absidia corymbifera* become resistant to most of the antifungal agents. Keeping in view the promising use of potentially metal-based antibacterial/antifungal/antiviral therapy that has provoked wide interest [5–14] into this diversified area, we, therefore, wish to report here some metal-based [(Co (II), Cu (II), Ni (II) and Zn (II)] compounds (**1–24**) incorporated with the novel isonicotinoylhydrazide Schiff's bases ($\mathbf{L}^1$–$\mathbf{L}^6$) (Figure 1) and their *in-vitro* antibacterial/antifungal

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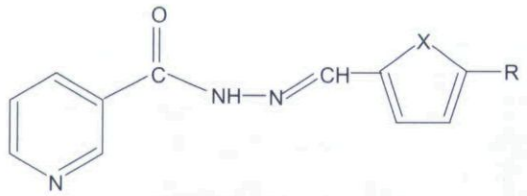

L^1: X = O; R = H L^2 : X = O; R = CH_3

L^3: X = O; R = NO_2 L^4 : X = S; R = H

L^5: X = S; R = CH_3 L^6 : X = S; R = NO_2

Figure 1. Proposed structures of the ligands.

activity. This group of compounds has been fully characterized on the basis of their IR, NMR, UV spectral and elemental analyses data and possesses a wide spectrum of antibacterial activity against *M. tuberculosis*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. dysenteriae*, *B. cereus*, *C. diphtheriae*, *S. aureus* and *S. pyogenes* and antifungal activity against *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani* and *C. glabrata* strains.

Experimental

Material and methods

Solvents used were of analytical grade and all metal (II) were used as chloride salts. IR spectra were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer and NMR spectra on a Perkin–Elmer 283B spectrometer. UV–Visible spectra were obtained in DMF on a Hitachi U-2000 double-beam spectrophotometer. Butterworth Laboratories Ltd (U.K.) carried out C, H and N analyses. Conductance of the metal complexes was determined in DMF on a Hitachi (Japan) YSI-32 model conduct meter. Magnetic measurements were carried out on solid complexes using the Gouy's method. Melting points were recorded on a Gallenkamp (U.K.) apparatus and are uncorrected. The complexes were analyzed for their metal contents by EDTA titration [15]. Antibacterial, antifungal and cytotoxic screening was done at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Preparation of Schiff's bases ($\mathbf{L}^1$–$\mathbf{L}^6$). General procedure for ($\mathbf{L}^1$). A solution of furyl-2-carboxaldehyde (0.01 mol, 1.12 mL) in ethanol (10 mL) was added to a magnetically stirred solution of isoniazid (0.01 mol, 1.37 g) in warm ethanol (30 mL). The mixture was refluxed for 3 h and the reaction monitored through TLC. After completion of the reaction (TLC analysis), it was cooled to afford a solid product. The solid residue was filtered, washed with cold ethanol, then with ether and dried. Crystallization from hot ethanol gave ($\mathbf{L}^1$). The same method was applied for the preparation of ($\mathbf{L}^2$–$\mathbf{L}^6$) by using the corresponding compounds, working under the same conditions with their same respective molar ratio.

N-(2-Furylmethylidene)nicotinohydrazide ($\mathbf{L}^1$). Yield: 78%. M. p. 175°C. IR (cm^{-1}, KBr): 3058 (m, NH), 1620 (m, C=O), 1588 (w, C=N), 1560 (s, C–O), 1535 (m, NNH). ^{1}H NMR (DMSO-d_6) δ: 6.9 (m, 1H, *H*-nict), 7.1 (m, 1H, *H*-nict), 7.7 (m, 1H, *H*-nict), 7.9 (m, 1H, *H*-nict), 9.8 (bs, 1 H, N*H*), 6.8 (s, 1H, C*H*=N), 8.6 (m, 1H, *H*-furyl), 8.1 (m, 1H, *H*-furyl), 8.8 (m, 1H, *H*-furyl). ^{13}C NMR (DMSO-d_6) δ: 133.1, 127.7, 125.6, 128.6, 129.2 (*C*-nict), 167.8 (*C*=O), 125.4 (H*C*=N), 150.3, 149.7, 133.5, 135.3 (*C*-furyl). Found: C, 61.7; H, 3.8; N, 19.1. $C_{11}H_9N_3O$ requires: C, 61.4; H, 4.2; N, 19.5%.

N-(5-Methyl-2-furylmethylidene)nicotinohydrazide ($\mathbf{L}^2$). Yield: 70%. M. p. 194°C. IR (cm^{-1}, KBr): 3058 (m, NH), 1620 (m, C=O), 1575 (w, C=N), 1560 (s, C–O), 1535 (m, NNH). ^{1}H NMR (DMSO-d_6) δ: 2.4 (s, 3H, C*H*$_3$), 6.9 (m, 1H, *H*-nict), 7.0 (m, 1H, *H*-nict), 7.6 (m, 1H, *H*-nict), 7.9 (m, 1H, *H*-nict), 9.7 (bs, 1 H, N*H*), 6.8 (s, 1H, C*H*=N), 8.6 (m, 1H, *H*-furyl), 8.2 (m, 1H, *H*-furyl), 8.8 (m, 1H, *H*-furyl). ^{13}C NMR (DMSO-d_6) δ: 15.2 (*C*H$_3$), 133.2, 127.7, 125.5, 128.6, 129.3 (*C*-nict), 167.8 (*C*=O), 125.5 (H*C*=N), 155.4, 149.7, 133.5, 135.2 (*C*-furyl). Found: C, 62.6; H, 4.9; N, 18.5. $C_{12}H_{11}N_2O_3$ requires: C, 62.9; H, 4.8; N, 18.3%.

N-(5-Nitro-2-furylmethylidene)nicotinohydrazide ($\mathbf{L}^3$). Yield: 68%. M. p. 184°C. IR (cm^{-1}, KBr): 3055 (m, NH), 1625 (m, C=O), 1575 (w, C=N), 1565 (s, C–O), 1535 (m, NNH). ^{1}H NMR (DMSO-d_6) δ: 6.9 (m, 1H, *H*-nict), 7.2 (m, 1H, *H*-nict), 7.7 (m, 1H, *H*-nict), 7.9 (m, 1H, *H*-nict), 9.8 (bs, 1 H, N*H*), 6.9 (s, 1H, C*H*=N), 8.7 (m, 1H, *H*-furyl), 8.3 (m, 1H, *H*-furyl), 8.8 (m, 1H, *H*-furyl). ^{13}C NMR (DMSO-d_6) δ: 133.4, 127.7, 125.6, 128.6, 129.4 (*C*-nict), 167.8 (*C*=O), 125.6 (H*C*=N), 167.5, 149.7, 133.6, 135.4 (*C*-furyl). Found: C, 51.2; H, 3.4; N, 21.2. $C_{11}H_8N_4O_4$ requires: C, 50.8; H, 3.1; N, 21.5%.

N-(2-Thienylmethylidene)nicotinohydrazide ($\mathbf{L}^4$). Yield: 75%. M. p. 188°C. IR (cm^{-1}, KBr): 3058 (m, NH), 1620 (m, C=O), 1575 (w, C=N), 1560 (s, C–S), 1535 (m, NNH). ^{1}H NMR (DMSO-d_6) δ: 6.7 (m, 1H, *H*-nict), 7.1 (m, 1H, *H*-nict), 7.5 (m, 1H, *H*-nict), 7.9 (m, 1H, *H*-nict), 9.7 (bs, 1 H, N*H*), 6.5 (s, 1H, C*H*=N), 8.5 (m, 1H, *H*-thienyl), 8.0 (m, 1H, *H*-thienyl), 8.7 (m, 1H, *H*-thienyl). ^{13}C NMR (DMSO-d_6) δ: 133.0, 127.5, 125.3, 128.4, 129.1 (*C*-nict), 167.8 (*C*=O), 125.2 (H*C*=N), 150.1, 149.5, 133.4, 135.1 (*C*-thienyl). Found: C, 57.4; H, 3.8; N, 18.5. $C_{11}H_9N_3OS$ requires: C, 57.1; H, 3.9; N, 18.2%.

N-(5-Methyl-2-thienylmethylidene)nicotinohydrazide (***L***^{5}). Yield: 69%. M. p. 192°C. IR (cm^{-1}, KBr): 3055 (m, NH), 1620 (m, C=O), 1575 (w, C=N), 1560 (s, C–S), 1535 (m, NNH). ^{1}H NMR (DMSO-d_6) δ: 2.5 (s, 3H, C*H*$_3$), 6.6 (m, 1H, *H*-nict), 7.2 (m, 1H, *H*-nict), 7.4 (m, 1H, *H*-nict), 7.8 (m, 1H, *H*-nict), 9.7 (bs, 1 H, N*H*), 6.5 (s, 1H, C*H*=N), 8.4 (m, 1H, *H*-thienyl), 8.1 (m, 1H, *H*-thienyl), 8.7 (m, 1H, *H*-thienyl). ^{13}C NMR (DMSO-d_6) δ: 15.3 (*C*H$_3$), 133.1, 127.5, 125.2, 128.4, 129.2 (*C*-nict), 167.8 (*C*=O), 125.3 (H*C*=N), 167.6, 149.5, 133.4, 135.1 (*C*-thienyl). Found: C, 59.1; H, 4.9; N, 17.5. $C_{12}H_{11}N_3OS$ requires: C, 58.8; H, 4.5; N, 17.1%.

N-(5-Nitro-2-thienylmethylidene)nicotinohydrazide (***L***^{6}). Yield: 67%. M. p. 189°C. IR (cm^{-1}, KBr): 3058 (m, NH), 1625 (m, C=O), 1575 (w, C=N), 1565 (s, C–S), 1535 (m, NNH). ^{1}H NMR (DMSO-d_6) δ: 6.7 (m, 1H, *H*-nict), 7.4 (m, 1H, *H*-nict), 7.6 (m, 1H, *H*-nict), 7.9 (m, 1H, *H*-nict), 9.6 (bs, 1 H, N*H*), 6.7 (s, 1H, C*H*=N), 8.5 (m, 1H, *H*-thienyl), 8.3 (m, 1H, *H*-thienyl), 8.7 (m, 1H, *H*-thienyl). ^{13}C NMR (DMSO-d_6) δ: 133.3, 127.5, 125.2, 128.5, 129.3 (*C*-nict), 167.9 (*C*=O), 125.3 (H*C*=N), 150.4, 149.5, 133.4, 135.3 (*C*-thienyl). Found: C, 48.1; H, 3.2; N, 20.0. $C_{11}H_8N_4O_3S$ requires: C, 47.8; H, 2.9; N, 20.3%.

*Preparation of metal (II) complexes (**1**–**24**). General procedure*

Cobalt (II) complex of ***L***^{1}. An ethanol solution (20 mL) of cobalt (II) chloride (5 mmol, 0.65 g) was added dropwise to **L**^{1} (10 mmol, 1.2 g) dissolved in absolute ethanol (30 mL), and heated for 1 h at 50°C with stirring. After cooling to room temperature a brown precipitate formed immediately. The product was separated by suction filtration, purified by washing with cold ethanol and then with ether. All other complexes were synthesized according to the same method.

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 using the agar well diffusion method [16]. 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 centers at least 24 mm. Recommended concentration of the test sample 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 with the standard drug, imipenum. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial/fungal strains.

Antifungal activity (in-vitro). Antifungal activity of all compounds was studied [17] against six fungal cultures. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspension 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 standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration (MIC). Compounds showing promising antibacterial activity were only selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion technique [16] by preparing discs containing 10, 25, 50 and 100 μg/ml of the compounds and applying the reported 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 [17] with a 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 DMSO. 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 DMSO only. The solvent was allowed to evaporate overnight. After two days, when the 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 number of survivors was counted. Data were analyzed by a

Table I. Physical and analytical data of the metal (II) complexes (**1**–**24**).

No	Metal complex/Mol. formula	D.P (°C)	B.M (μ_{eff})	C, H, N; Calc. (Found) %	λ_{max} (cm^{-1})	IR (cm^{-1})
1	$[Co(L^1)_2Cl_2]$[557.8] $C_{22}H_{16}CoCl_2N_6O_4$	318–320	4.6	47.3 2.9 15.1 (47.8)(2.9)(15.2)	8615,17520, 30115	1565 (C=N), 1515 (NNH), 460 (M–O), 395, (MN), 315 (M–Cl)
2	$[Co(L^2)_2Cl_2]$[585.8] $C_{24}H_{20}CoCl_2N_6O_4$	322–324	4.8	49.2 3.4 14.3 (49.3)(3.2)(14.4)	8895,17665, 29980	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
3	$[Co(L^3)_2Cl_2]$[647.8] $C_{22}H_{14}CoCl_2N_8O_8$	335–337	4.6	40.8 2.2 17.3 (41.2)(2.3)(17.0)	8790,17570, 29995	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
4	$[Co(L^4)_2Cl_2]$[711.9] $C_{22}H_{14}CoCl_2N_8O_6S_2$	320–322	4.7	37.1 2.0 15.7 (37.5)(2.3)(15.3)	8815,17595, 30105	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
5	$[Co(L^5)_2Cl_2]$[619.9] $C_{24}H_{22}CoCl_2N_6O_2S_2$	326–328	4.7	46.5 3.5 13.6 (46.6)(3.8)(13.3)	8675,17615, 30000	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
6	$[Co(L^6)_2Cl_2]$[589.9] $C_{22}H_{16}CoCl_2N_6O_2S_2$	316–318	4.8	44.8 2.7 14.2 (44.6)(2.9)(14.3)	8855,17630, 30111	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
7	$[Cu(L^1)_2]Cl_2$ [562.4] $C_{22}H_{16}CuCl_2N_6O_4$	322–324	1.4	46.9 2.8 14.9 (46.6)(2.5)(15.3)	15215,19260, 30310	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N)
8	$[Cu(L^2)_2]Cl_2$ [590.4] $C_{24}H_{20}CuCl_2N_6O_4$	322–324	1.6	48.8 3.4 14.2 (48.6)(3.0)(14.5)	15355,19675, 30175	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N)
9	$[Cu(L^3)_2]Cl_2$ [652.4] $C_{22}H_{14}CuCl_2N_8O_8$	318–320	1.3	40.5 2.3 17.2 (40.7)(2.5)(17.4)	15265, 19285, 30355	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N)
10	$[Cu(L^4)_2]Cl_2$ [716.5] $C_{22}H_{14}CuCl_2N_8O_6S_2$	324–326	1.5	36.8 2.0 15.6 (36.3)(2.5)(15.4)	15280, 19445, 30200	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N)
11	$[Cu(L^5)_2]Cl_2$ [624.5] $C_{24}H_{22}CuCl_2N_6O_2S_2$	328–330	1.5	46.1 3.5 13.4 (46.5)(3.8)(13.5)	15310, 19550, 30285	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
12	$[Cu(L^6)_2]Cl_2$ [594.5] $C_{22}H_{16}CuCl_2N_6O_2S_2$	320–324	1.3	44.4 2.7 14.1 (44.1)(2.5)(14.3)	15345, 19615, 30245	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N)
13	$[Ni(L^1)_2Cl_2]$[557.6] $C_{22}H_{16}NiCl_2N_6O_4$	330–332	3.4	47.3 2.9 19.0 (47.6)(2.7)(19.4)	10585, 16345, 29480	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
14	$[Ni(L^2)_2Cl_2]$[585.6] $C_{24}H_{20}NiCl_2N_6O_4$	325–327	3.6	49.2 3.4 14.3 (49.5)(3.2)(14.7)	10150, 16455, 29965	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
15	$[Ni(L^3)_2Cl_2]$[647.6] $C_{22}H_{14}NiCl_2N_8O_8$	328–330	3.5	40.8 2.2 17.3 (40.5)(2.0)(17.1)	10275, 16370, 29565	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
16	$[Ni(L^4)_2Cl_2]$[711.7] $C_{22}H_{14}NiCl_2N_8O_6S_2$	323–325	3.4	37.1 2.0 15.7 (37.2)(2.5)(15.3)	10465, 16395, 29615	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
17	$[Ni(L^5)_2Cl_2]$[619.7] $C_{24}H_{22}NiCl_2N_6O_2S_2$	326–328	3.6	46.5 3.6 13.6 (46.2)(3.2)(13.7)	10525, 16410, 29595	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
18	$[Ni(L^6)_2Cl_2]$[589.7] $C_{22}H_{16}NiCl_2N_6O_2S_2$	325–328	3.4	44.8 2.7 14.2 (45.1)(2.5)(12.4)	10440, 16435, 29815	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
19	$[Zn(L^1)_2Cl_2]$[564.3] $C_{22}H_{16}ZnCl_2N_6O_4$	328–330	Dia	46.8 2.8 14.9 (46.3)(2.5)(14.7)	28540	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
20	$[Zn(L^2)_2Cl_2]$[592.3] $C_{24}H_{20}ZnCl_2N_6O_4$	320–322	Dia	48.6 3.4 14.2 (48.9)(3.0)(14.4)	29385	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
21	$[Zn(L^3)_2Cl_2]$[654.3] $C_{22}H_{14}ZnCl_2N_8O_8$	318–320	Dia	40.4 2.1 17.1 (40.9)(2.5)(17.4)	28770	1565 (C=N), 1515 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)

Table I – *continued*

No	Metal complex/Mol. formula	D.P (°C)	B.M (μ_{eff})	C, H, N; Calc. (Found) %	λ_{max} (cm^{-1})	IR (cm^{-1})
22	$[Zn(L^4)_2]Cl_2$][718.4] $C_{22}H_{14}ZnCl_2N_8O_6S_2$	324–326	Dia	36.8 1.9 15.6 (36.4)(2.2)(15.4)	29210	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
23	$[Zn(L^5)_2]Cl_2$][626.4] $C_{24}H_{22}ZnCl_2N_6O_2S_2$	322–324	Dia	46.0 3.5 13.4 (46.4)(3.8)(13.8)	29195	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)
24	$[Zn(L^6)_2]Cl_2$][596.4] $C_{22}H_{16}ZnCl_2N_6O_2S_2$	325–327	Dia	44.3 2.7 14.1 (44.5)(2.9)(14.3)	28905	1565 (C=N), 1510 (NNH), 460 (M–O), 395 (M–N), 315 (M–Cl)

Finney computer program to determine the LD_{50} values. [18]

Result and discussion

Chemistry

The Schiff's bases ($\mathbf{L}^1$–$\mathbf{L}^6$) (Figure 1) were prepared by refluxing an equimolar amount of isonicotinoyl hydrazide with 2-furylcarboxaldehyde, 5-methyl-2-furylcarboxaldehyde, 5-nitro-2-furylcarboxaldehyde, 2-thiphenecarboxaldehyde, 5-methyl-2-thiophenecarboxal-dehyde and 5-nitro-2-thiophenecarboxaldehyde respectively, in ethanol (30 mL). The structures of these Schiff's bases formed were established by IR, NMR, and microanalytical data. These Schiff's bases were further used for the complexation reaction with Co (II), Cu (II) Ni (II) and Zn (II) metal ions. All of the newly synthesized metal complexes (**1**–**24**) (Table I) were air and moisture stable. They were prepared by the stoichiometric reaction of the corresponding metal (II) salts (as chlorides) and the respective Schiff's bases in the molar ratio M:L of 1:2. The complexes are amorphous solids, which decompose above 200°C. They are insoluble in common organic solvents such as chloroform, acetone, ethanol and methanol, but soluble in DMSO and DMF. Molar conductance values of the soluble complexes in DMF showed values ($15–21\ ohm^{-1}\,cm^2\,mol^{-1}$), indicating [19] Co (II), Ni (II) and Zn (II) complexes to be non-electrolytes and ($88–92\ ohm^{-1}\,cm^2\,mol^{-1}$) for Cu (II) complexes to be electrolytic in nature. The elemental analyses data of the Schiff's bases (reported as in experimental) and their complexes (Table I) is compatible with the structures of the ligands as shown in Figure 1 and with that of the formulas of the complexes as $[M(L)_2Cl\text{-}_2]$ where [M=Co, Ni or Zn] and $[M(L)_2]Cl_2$ [M=Cu]. The suggested structures of the ligands and their complexes are shown in Figures 1 and 2.

Infrared spectra. IR spectra of the ligands showed the absence of bands at 1735 and 3420 cm^{-1} due to carbonyl ν(C=O) and $\nu(NH_2)$ stretching vibrations and instead, a new band appeared at ~1588 cm^{-1} assigned [20] to the azomethine ν(HC=N) linkage. This suggested that amino and aldehyde moieties of the starting reagents have been converted into their corresponding Schiff's bases (Figure 1). The ν(NH)-amide, ν(C=O)-amide and ν(NNH)-imino stretching frequencies were present respectively, at 3058, 1616 and 1530 cm^{-1}, respectively. A comparison[21] of the IR spectra of the Schiff's bases to their metal (II) chelates (Table I), indicated that the Schiff's bases are coordinated to the metal atom mainly in two ways, thus the ligands are acting in a bidentate manner. The band appearing at 1588 cm^{-1} due to the azomethine

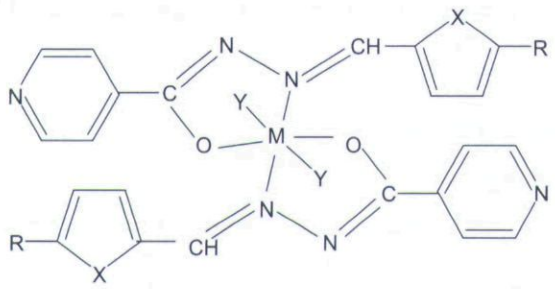

Figure 2. Proposed structures of the metal (II) complexes.

group was shifted to lower frequency by $\sim 20\,cm^{-1}$ indicating [20] participation of the azomethine nitrogen in the complexation. A new medium-strong band appearing at $460\,cm^{-1}$ is assigned [22] to ν(M–O). This demonstrates that oxygen of the C=O-amide has formed a coordinative bond with the metal ions in an enolic form. A weak band at $395\,cm^{-1}$ is assigned to ν(M–N). This further confirms that the nitrogen of the HNN-imino group bonds to the metal atom. Furthermore, a weak band at $315\,cm^{-1}$ was observed in the spectra of the Co (II), Ni (II) and Zn (II) complexes suggesting [23] chloride atoms to be coordinated with the metal ion showing an octahedral geometry. However, in the spectra of the Cu (II) complexes this band was not observable, indicating that the chloride atoms are not coordinated with the metal ion but remain outside the coordination sphere thus showing a square-planar geometry. All of the data establish that a conjugate chelate ring formed by ligand enolization exists in the complexes.

NMR spectra. The NMR spectra of the free ligands were determined in DMSO-d_6. The ^{1}H NMR spectral data are reported along with the possible assignments. All the protons were found to be in their expected region [24,25]. The conclusions drawn from these studies lend further support to the mode of bonding

Table II. *In-vitro* antibacterial activity data for the ligands ($\mathbf{L}^1$–$\mathbf{L}^6$) and metal (II) complexes (**1**–**24**).

	Diameter of zones showing complete inhibition of growth (mm)									
Compound	*(a)*	*(b)*	*(c)*	*(d)*	*(e)*	*(f)*	*(g)*	*(h)*	*(j)*	*(k)*
$\mathbf{L}^1$	23	15	14	16	7	15	6	5	14	12
$\mathbf{L}^2$	22	18	12	17	5	18	8	10	12	13
$\mathbf{L}^3$	24	18	16	18	6	15	12	13	14	15
$\mathbf{L}^4$	25	15	18	19	5	18	15	14	15	17
$\mathbf{L}^5$	21	12	12	15	6	14	6	10	12	12
$\mathbf{L}^6$	22	14	10	15	5	14	6	10	11	15
1	28	22	21	20	10	22	18	15	18	19
2	30	20	20	22	9	21	16	16	17	20
3	28	22	21	20	8	21	19	17	20	20
4	27	20	19	20	9	22	15	15	21	19
5	28	21	21	20	7	20	18	14	18	20
6	28	22	21	20	6	20	15	15	20	20
7	29	20	22	24	10	22	16	14	18	21
8	30	25	20	22	11	22	18	18	19	21
9	32	23	20	23	9	20	16	15	20	20
10	30	22	20	24	10	21	18	15	18	20
11	28	22	22	22	11	23	15	16	18	19
12	29	20	20	22	11	20	14	15	18	19
13	23	20	22	25	7	22	15	15	18	19
14	22	22	22	23	8	22	18	16	19	18
15	25	23	22	24	8	22	15	15	18	18
16	24	22	20	23	8	21	15	14	18	19
17	22	20	22	22	8	21	18	12	18	18
18	25	22	20	22	7	21	19	15	18	18
19	29	20	22	22	9	21	18	14	20	20
20	30	22	21	22	10	22	16	15	17	19
21	30	22	19	22	11	20	15	15	18	20
22	32	20	19	22	10	23	15	14	17	20
23	31	21	20	22	9	23	16	12	18	19
24	31	24	20	22	10	23	14	14	20	19
Imipenum	30	30	25	30	32	30	30	25	30	32

Ligand: >15 mm = significant activity; 7–14 mm = moderate activity; <7 mm = weak activity. *(a)* = *M. tuberculosis (b)* = *E. coli, (c)* = *K. pneumoniae, (d)* = *P. mirabilis, (e)* = *P. aeruginosa, (f)* = *S. typhi, (g)* = *S. dysenteriae, (h)* = *B. cereus, (j)* = *C. diphtheriae, (k)* = *S. pyogenes,* (m) = *S. aureus.* Imipenum = standard drug.

Table III. *In-vitro* antifungal activity data for the ligands ($\mathbf{L}^1$–$\mathbf{L}^6$) and metal (II) complexes (**1**–**24**).

Compound	Diameter of zones showing complete inhibition of growth (mm)					
	(a)	*(b)*	*(c)*	*(d)*	*(e)*	*(f)*
$\mathbf{L}^1$	15	17	11	16	12	10
$\mathbf{L}^2$	14	12	14	14	15	13
$\mathbf{L}^3$	15	15	12	15	10	14
$\mathbf{L}^4$	14	15	11	15	10	14
$\mathbf{L}^5$	15	14	11	14	12	14
$\mathbf{L}^6$	16	15	11	15	12	12
1	17	15	15	18	22	17
2	17	12	15	18	20	19
3	19	24	15	20	18	18
4	17	12	14	20	20	19
5	20	11	15	18	06	20
6	20	18	14	20	20	18
7	20	18	16	21	18	19
8	17	16	16	18	18	19
9	19	15	18	20	15	18
10	18	16	15	18	20	15
11	20	15	15	20	18	19
12	17	14	18	20	18	19
13	19	16	14	21	20	17
14	20	17	18	18	18	17
15	19	18	16	20	18	17
16	17	18	16	18	20	16
17	17	18	18	18	21	18
18	18	14	15	20	22	20
19	19	13	16	20	18	18
20	20	15	16	20	20	17
21	19	11	20	15	21	18
22	17	16	18	20	22	20
23	19	14	20	18	18	19
24	17	11	16	20	18	19
Miconazole	25	20	25	25	25	25
Amphotericin B	30	25	30	25	25	30

Ligand: >14 mm = significant activity; 7–13 mm = moderate activity; <7 mm = weak activity. *(a)* = *T. longifusus*, *(b)* = *C. albicans*, *(c)* = *A. flavus*, *(d)* = *M. canis*, *(e)* = *F. solani*, *(f)* = *C. glabrata*. Miconazole and Amphotericin B = standard drugs.

Table IV. Brine shrimp bioassay data for the ligands ($\mathbf{L}^1$–$\mathbf{L}^6$) and their metal (II) complexes (**1**–**24**).

Compound	LD_{50} (μg/ml)
$\mathbf{L}^1$	>1000
$\mathbf{L}^2$	>1000
$\mathbf{L}^3$	423
$\mathbf{L}^4$	>1000
$\mathbf{L}^5$	>1000
$\mathbf{L}^6$	365
1	>1000
2	>1000
3	>1000
4	>1000
5	>1000
6	>1000
7	>1000
8	>1000
9	>1000
10	430
11	354
12	>1000
13	>1000
14	>1000
15	>1000
16	>1000
17	>1000
18	>1000
19	>1000
20	>1000
21	>1000
22	312
23	406
24	>1000

discussed in their IR spectra. The number of protons calculated from the integration curves, and those obtained from the values of the expected CHN analyses agree. It was also observed that DMSO did not have any coordinating effect on the spectra of the ligands or their metal complexes.

Magnetic moments and electronic spectra. The nature of the ligand field around the metal ion and the geometry of the metal complexes have been deduced from the electronic spectra and magnetic moment data of the complexes (Table I). The room temperature magnetic moment of the solid cobalt (II) complexes was found in the range 4.6–4.8 B.M, indicative [26] of three unpaired electrons per Co (II) ion in an ideal octahedral environment. Also, the magnetic moment values (1.3–1.6 B.M) for the copper (II) are indicative of anti-ferromagnetic spin–spin interaction through molecular association [27] for square-planar geometry. The nickel (II) complexes showed μ_{eff} values in the range 3.4–3.6 B.M, corresponding [27] to two unpaired electrons per Ni (II) ion for their six-coordinated configuration. The electronic spectra of the Co (II) complexes showed three bands observed at 8,615–8,895, 17,520–17,665 and 29,980–30,115 cm^{-1} which may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}(F)$, $^4T_{1g} \rightarrow {}^3A_{2g}(F)$ and $^4T_{1g} \rightarrow {}^4T_{1g}(P)$ transitions, respectively, and are suggestive[28] of an octahedral geometry around the cobalt ions. The electronic spectra of the Cu (II) complexes showed two low-energy weak bands at 15,215–15,355 and 19,260–19,675 cm^{-1} and a strong high-energy band at 30,175–30,310 cm^{-1} and may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively, for their square-planar geometry. The strong high-energy band, in turn, is assigned to metal → ligand charge transfer. The Ni (II) complexes exhibited three spin-allowed bands at 10,150–10,585, 16,345–16,455, and 29,480–29,965 cm^{-1} assignable [29], respectively, to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)(\nu_3)$ which are characteristic of their octahedral geometry. The diamagnetic Zn (II) complexes exhibited only a high-intensity band at 28,540–29,385 cm^{-1} assigned [30] to ligand–metal charge transfer.

Table V. Minimum inhibitory concentration (μg/ml) of the ligands and their metal complexes.

Compound	*(a)*	*(b)*	*(c)*	*(d)*	*(e)*	*(f)*	*(g)*	*(h)*	*(j)*	*(k)*
$\mathbf{L}^1$	10	–	–	>100	–	–	–	–	–	–
$\mathbf{L}^2$	10	–	–	>100	10	10	–	–	–	–
$\mathbf{L}^3$	10	>100	–	10	>100	–	–	–	–	–
$\mathbf{L}^4$	10	–	–	>100	10	10	–	–	–	10
$\mathbf{L}^5$	–	–	–	–	10	–	–	–	–	–
$\mathbf{L}^6$	>100	–	–	–	–	–	–	–	–	–
1	10	>100	>100	>100	10	10	10	–	>100	25
2	10	25	–	25	>100	>100	>100	>100	10	10
3	10	>100	>100	>100	>100	>100	>100	>100	10	10
4	–	>100	–	>100	10	>100	–	–	25	10
5	10	>100	>100	–	10	25	10	–	10	10
6	>100	>100	25	>100	10	10	–	–	>100	>100
7	10	>100	>100	25	>100	>100	>100	–	10	>100
8	25	>100		– > 100	>100	10	>100	10 > 100	10	
9	10	–	>100	>100	>100	>100	>100	10	–	>100
10	>100	>100	–	>100	>100	10	>100	–	>100	>100
11	10	–	>100	25	>100	10	–	>100	10	>100
12	>10	–	–	>100	25	>100	–	–	10	>100
13	10	25	25	>100	>100	>100	–	–	>100	>100
14	>100	>100	–	>100	>100	10	>100	>100	>100	10
15	10	25	–	>100	>100	>100	–	>100	>100	25
16	>10	>100	>100	25	>100	10	–	–	>100	>100
17	25	10	>100	>100	>100	10	>100	–	>100	>100
18	25	>100	–	>100	10	>100	25	–	10	>100
19	10	>100	–	>100	10	>100	>100	–	10	10
20	10	10	>100	>100	>100	>100	>100	–	>100	>100
21	>10	>100	>100	>100	>100	–	–	–	>100	>100
22	10	10	25	>100	>100	>100	–	–	>100	25
23	10	10	>100	25	25	>100	>100	–	25	>100
24	>10	10	>100	>100	>100	>100	–	–	>100	>100

(a) = *M. tuberculosis*, *(b)* = *E. coli*, *(c)* = *K. pneumoniae*, *(d)* = *P. mirabilis*, *(e)* = *P. aeruginosa*, *(f)* = *S. typhi*, *(g)* = *S. dysenteriae*, *(h)* = *B. cereus*, *(j)* = *C. diphtheriae*, *(k)* = *S. pyogenes*. No activity = –.

On the basis of the above observations, it is suggested that the Co (II), Ni (II) and Zn (II) complexes show an octahedral geometry in which the two Schiff's bases act as bidentate and accommodate themselves around the metal atom in such a way that a stable chelate ring of the complex is formed and two chloride atoms also coordinate to the metal ion forming a stable octahedral structure of the complexes. The Cu (II) complexes give a square-planar geometry by only coordination of the bidentate Schiff's bases without coordination of the chloride ions that stay uncoordinated outside the coordination sphere.

Antibacterial bioassay

All compounds were tested against *M. tuberculosis*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. dysenteriae*, *B. cereus*, *C. diphtheriae*, *S. aureus* and *S. pyogenes* bacterial strains (Table II) according to literature protocol [16]. The results were compared with those of the standard drug imipenum. All ligands were found potentially active against one or more bacterial strains. Cobalt (II), copper (II), nickel (II) and zinc (II) metal complexes (**1**–**24**) of these synthesized ligands ($\mathbf{L}^1$–$\mathbf{L}^6$) were also screened against the same bacterial strains. It was evident that overall potency of the uncoordinated compounds/ligands was enhanced on coordination with the metal ions.

Antifungal bioassay

Antifungal screening of all compounds was carried out against *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani* and *C. glabrata* fungal strains according to the literature protocol [17]. The results were compared with the standard drugs miconazole and amphotericin B. These results illustrated in Table III indicate that all ligands were active against one or more fungal species however, the metal (II) complexes (**1**–**24**) of these compounds relatively showed much enhanced activity as compared to the uncoordinated compounds.

Cytotoxic bioassay

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer et al. [31,17]. Only ligands $\mathbf{L}^3$ and $\mathbf{L}^6$ and the Cu (II) and Ni (II) metal complexes (**10**, **11**, **22** & **23**) displayed (Table IV) cytotoxic activity against *A. salina*, while the other compounds gave values of

$LD_{50} > 1000$ in this assay, and therefore, can be considered to be inactive in this assay.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the disc diffusion method [16]. MIC was the lowest concentration of a substance at which the inhibition of growth occurred. The MIC of these compounds varied from 10–100 μg/ml. The results Table V indicated that these compounds proved to be the most active by inhibiting the growth of the tested organisms at 10 μg/ml concentrations.

Some of the compounds generally showed good antibacterial activity against two or four and, moderate and insignificant activity against one or two bacterial species. However, they showed moderate antifungal activity against most of the species. It was evident from the data that this activity significantly increased on coordination. This enhancement in the activity of ($\mathbf{L}^1$–$\mathbf{L}^6$) may also be rationalized on the basis of their structures by mainly possessing an additional azomethine (HC=N) bond. It has been suggested that the ligands with nitrogen and oxygen donor systems inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions on coordination. Moreover, coordination reduces the polarity [32,33] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups within the chelate ring system, which is mainly formed during chelation. This process, in turn, increases the lipophilic nature of the central metal atom, which favors its permeation more efficiently through the lipoid layer of the microorganism [34–37] thus making the chelate compounds bacteriostatic and fungistatic.

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