APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2005; **19**: 1207–1214
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.944

Organometallic compounds with biologically active molecules: *in vitro* antibacterial and antifungal activity of some 1,1′-(dicarbohydrazono) ferrocenes and their cobalt(II), copper(II), nickel(II) and zinc(II) complexes

Zahid H. Chohan[1]* and Claudiu T. Supuran[2]

[1]Department of Chemistry, Bahauddin Zakariya University, Multan, Pakistan
[2]University of Florence, Dipartmento di Chimica, Laboratorio di Chimica Bioinorganica, Via della Lastruccia, Rm. 188, Polo Scientifico, 50019, Sesto Fiorentino, Florence, Italy

Received 24 February 2005; Accepted 18 April 2005

Some 1,1′-(dicarbohydrazono) ferrocenes have been prepared by condensing 1,1′-diacetylferrocene with either 2-furoic hydrazide, 2-thiophenecarboxylic hydrazide or 2-salicylic hydrazide. All the ligands synthesized were characterized by IR and NMR spectroscopy and elemental analysis data (carbon, hydrogen, nitrogen) and then were used as ligands to react with cobalt(II), copper(II), nickel(II) and zinc(II) metals as chlorides to afford metal complexes having the general formula $M(L)Cl_2$. IR and electronic spectral data, magnetic moment and elemental analyses were used in the structural investigation of the metal complexes synthesized. The ligands synthesized and their metal(II) complexes have been screened for their *in vitro* antibacterial activity against *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 than the uncomplexed ligands. However, the potency of all the ligands synthesized and their metal complexes was lower than that of the standard drugs.

KEYWORDS: 1,1′-(dicarbohydrazono) ferrocenes; metal complexes; antibacterial; antifungal

INTRODUCTION

It is well known[1–3] that some drugs have increased activity when administered as metal complexes. Carbohydrazones are a special class of azomethine[4] that act as ionic or neutral moieties. The β-nitrogen present in these compounds coordinated to the metal atom has an interesting stereochemistry, whereas the α-nitrogen remains uncoordinated. On the other hand, the remaining oxygen atom can form a covalent bond with the metal atom. Many reports on the chemistry and potential antitumour,[5] antibacterial,[6] antiviral,[7–9] antifungal,[10] antipesticidal[11] and antimalarial[12] activities of transition-metal complexes of thiosemicarbazone/semicarbazone are available.[13] However, only a few reports have appeared on the biocidal activity[14–16] of organometallic compounds such as ferrocene-derived carbohydrazones and their transition-metal complexes. Keeping in view the significance of this area in designing ferrocene-containing biologically active compounds of a typical organometallic nature and their metal complexes, we have previously reported[17–20] some ferrocene-derived mono- and/or di-substituted biologically active compounds and their various transition-metal complexes. In continuation of this, we report here on the preparation of 1,1′-(dicarbohydrazono) ferrocenes (Fig. 1) and their use as ligands for the preparation of their cobalt(II), copper(II), nickel(II) and zinc(II) metal complexes. The ligands synthesized ($\mathbf{L^1} - \mathbf{L^3}$) and their metal complexes (**1–12**) have been further investigated for their *in vitro* antibacterial activity

*Correspondence to: Zahid H. Chohan, Department of Chemistry, Bahauddin Zakariya University, Multan, Pakistan.
E-mail: zchohan@mul.paknet.com.pk

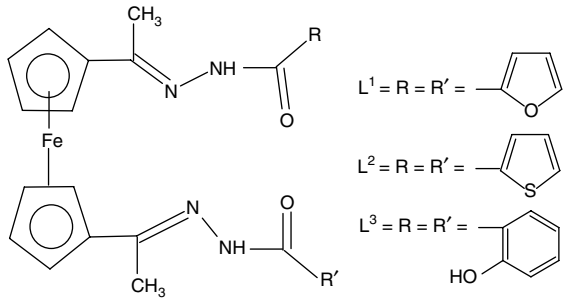

Figure 1. Proposed structure of the ligands.

against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Bacillus cereus*, *Corynebacterium diphtheriae*, *Staphylococcus aureus* and *Streptococcus pyogenes* and for *in vitro* antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberata* strains. Our interest in this research is based on the expectation that the cyclopentadienylmetal moiety in ferrocene may cause electronic effects on the coordination behaviour of the donor centres of these ligands that may influence the *in vitro* antibacterial/antifungal properties of such organometallic-based compounds.

EXPERIMENTAL

Material and Methods

Solvents used were analytical grade; all the metal(II) salts used were chlorides. IR spectra were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer. NMR spectra were recorded on Perkin–Elmer 283B spectrometer. UV–visible spectra were obtained in dimethylformamide (DMF) on a Hitachi U-2000 double-beam spectrophotometer. Butterworth Laboratories Ltd carried out carbon, hydrogen and nitrogen analyses. Conductance of the metal complexes was determined in DMF on a Hitachi (Japan) YSI-32 model conductance meter. Magnetic measurements were carried out on solid complexes using the Gouy method. Melting points were recorded on a Gallenkamp (UK) apparatus and are not corrected. The complexes were analysed for their metal contents by EDTA titration.[21]

Preparation of ligands (L^1–L^3) and metal(II) complexes (1–12)

Preparation of ligands

To a stirred warm ethanol solution (30 ml) of 2-furoic hydrazide (1.2 g, 0.02 mol) was added 1,1′-diacetylferrocene (2.7 g, 0.01 mol) in ethanol (50 ml). The mixture was refluxed for 8 h. The completion of reaction was monitored through thin-layer chromatography. After completion of the reaction, it was cooled to afford a solid product. The solid residue was filtered, washed with cold ethanol, then with diethyl ether and dried. Crystallization from hot ethanol : dichloromethane (70 : 30) gave ($\mathbf{L}^1$). The same method was applied for the preparation of $\mathbf{L}^2$ and $\mathbf{L}^3$ by using the corresponding hydrazides, working in the same conditions with their same respective molar ratios.

Preparation of metal(II) complexes (1–12)

For the preparation of the metal(II) complexes (**1–12**) from their respective metal(II) salts, a solution (20 ml) of the corresponding ligand (0.01 mol) in hot ethanol was added to a stirred solution of metal(II) chloride (0.01 mol) in ethanol (25 ml). The mixture was refluxed for 2 h and then cooled to room temperature, whereupon it solidified. The solid thus obtained was filtered, washed with ethanol, then with diethyl ether and dried in air. Crystallization from ethanol : dichloromethane (70 : 30) gave the desired metal complex.

Biological activity

Antibacterial activity (in vitro)

All the ligands synthesized ($\mathbf{L}^1 - \mathbf{L}^3$) and their corresponding metal(II) complexes (**1–12**) were screened *in vitro* for their antibacterial activity against *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. dysenteriae*, *B. cereus*, *C. diphtheriae*, *S. aureus and S. pyogenes* using the agar well-diffusion method.[21] Bacterial inoculums, 2 to 8 h old, containing approximately 10^4–10^6 colony forming units (CFU)/ml were used in these assays. Wells were dug in the media using a sterile metallic borer with centres of at least 24 mm. A recommended concentration (100 µl) of the test sample (1 mg ml^{-1} in dimethylsulfoxide (DMSO)) was introduced in the respective wells. Other wells supplemented with imipenum (1 mg ml^{-1} in DMSO) the reference antibacterial drug, 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 (in millimetres) of zones showing complete inhibition. Growth inhibition was compared with the standard drug. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions of DMSO alone; no activity against any bacterial strains was apparent.

Antifungal activity (in vitro)

Antifungal activities of all compounds were studied against six fungal cultures. Sabouraud dextrose agar (Oxoid, Hampshire, UK) was seeded with a 10^5 CFU ml^{-1} fungal spore suspension and transferred to Petri plates. Discs soaked in 20 ml (10 µg ml^{-1} in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32 °C for 7 days. The results were recorded as zones of inhibition (in millimetres) and compared with the standard drugs miconazole and amphotericin B.

MIC

Compounds showing promising activity were selected for MIC studies. The MIC was determined using the disc

diffusion technique by preparing discs containing 10, 25, 50 and 100 μg ml^{-1} of the compounds and applying the reported protocol.[21]

RESULTS AND DISCUSSION

Chemistry

1,1′-Diacetyl moieties of ferrocene were condensed with the corresponding hydrazides in 1:2 molar ratio, leading to a new series of Schiff base ligands (**L^1**–**L^3**; Fig. 1). The structures of these synthesized ligands were established with the help of IR, NMR and microanalytical data (Tables 1 and 2). The compounds synthesized were further used as ligands to prepare their cobalt(II), copper(II), nickel(II) and zinc(II) metal complexes (**1–12**) by the stoichiometric reaction of the corresponding ligand with the respective metal(II) chlorides in a 1:1 molar ratio. The metal complexes were characterized by IR, NMR, UV–visible, molar conductance, magnetic moment and elemental analyses data. They are all air- and moisture-stable and are intensely coloured amorphous solids that decompose without melting. The molar conductance values of the soluble copper(II) complexes in DMF (10^3 M solution at 25 °C) had high values (92–97 $\Omega^{-1}cm^{-2}$ mol^{-1}), suggesting that they are electrolytic in nature.[22] The molar conductance values were lower (21–25 $\Omega^{-1}cm^{-2}mol^{-1}$) in the case of the cobalt(II), nickel(II) and zinc(II) complexes, also suggestive of an electrolytic nature.[22] Efforts to grow good crystals of the ligands and their metal complexes for X-ray diffraction studies were unsuccessful.

IR spectra

Selected IR spectra of the ligands and their metal complexes, along with their tentative assignments, are reported in Tables 1 and 4. The IR spectra of the free ligands were compared with those of the complexes to confirm their authenticity and the coordination behaviour of the ligands with the metal ions. The spectra of the free Schiff base ligands show the presence of a strong band at 1725 cm^{-1} due to ν(C═O) and the appearance of a new band at 1610 cm^{-1} attributed[23] to the absorption of the azomethine ν(C═N) group that emerged due to condensation of the terminal amino (–NH_2) group of the hydrazide and –C═O groups of the acetylferrocene moieties. A medium strong band at 3120 cm^{-1} was attributed[24] to ν(N–NH) found in the spectra of the free ligands, which further strengthens the evidence for formation of the azomethine linkage and, hence, preparation of Schiff base ligands. The absorption due to ν(C═N) that appeared in the free Schiff base ligands at 1610 cm^{-1} shifted to a lower region in the spectra of all the complexes by 10 – 20 cm^{-1} (i.e. 1590–1600 cm^{-1}), showing the coordination of the azomethine nitrogen to the metal. The band located at 1725 cm^{-1} in all the ligands attributed[25] to furoic, thiophenoic and salicyloic ν(–C═O) moieties also moved to the lower frequency side by 15–20 cm^{-1} in the spectra of the metal complexes, suggesting coordination of the furoic-, thiophenoic- and salicyloic-oxygen with the metal atom. Furthermore, two new bands found at 425 and 485 cm^{-1} in the spectra of all the metal complexes (but not present in the ligands) confirmed involvement of the azomethine-nitrogen and C═O with the metal atom. Also, a new band was observed in the far-IR region at 315 cm^{-1}, assigned[26] to the coordination of ν(M–Cl) in the cobalt(II), nickel(II) and zinc(II) complexes, whereas in the spectra of the copper(II) complexes this band was not observed. This indicates an octahedral geometry for the cobalt(II), nickel(II) and zinc(II) complexes (Fig. 2a) and a square-planar geometry for the copper(II) complexes (Fig. 2b). Accordingly, the above-mentioned data suggest that the ligands behave in a tetradentate fashion towards all metals

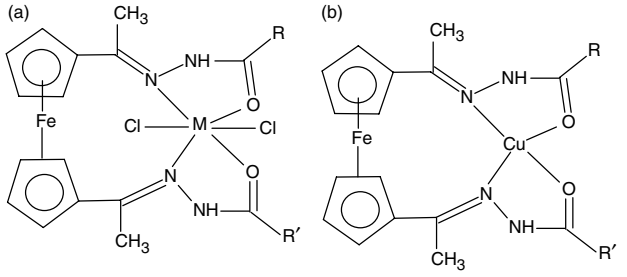

Figure 2. Proposed structure of the metal(II) complexes (M = Co(II), Ni(II), Zn(II)).

Table 1. Physical, spectral and analytical data of the ligands **L^1**–**L^3**

Ligand, molecular formula	M.p. (°C)	IR (cm^{-1})	Analysis, calc. (found) (%) C	H	N	Yield (%)
L^1 [485.9], $C_{24}H_{22}FeN_4O_4$	172	3120 (m, N–NH), 1725 (s, C═O), 1610 (s, C═N)	59.3 (59.6)	4.5 (4.4)	11.5 (11.9)	65
L^2 [518.0], $C_{24}H_{22}FeN_4O_2S_2$	164	3120 (m, N–NH), 1725 (C═O), 1610 (s, C═N)	55.6 (55.2)	4.2 (4.3)	10.8 (10.5)	62
L^3 [537.9], $C_{28}H_{26}FeN_4O_4$	158	3315 (OH), 3125 (m, N–NH), 1730 (C═O), 1610 (s, C═N)	62.5 (62.8)	4.8 (4.5)	10.4 (10.1)	63

Table 2. ^{1}H and ^{13}C NMR data of the ligands $\mathbf{L^1}$–$\mathbf{L^3}$ and their zinc(II) complexes **10–12**

	^{1}H NMR (DMSO-d_6) (ppm)	^{13}C NMR (DMSO-d_6) (ppm)
$\mathbf{L}^1$	2.2 (s, 6H, CH_3), 4.2–4.5 (m, 4H, ferrocenyl), 4.8–5.1 (m, 4H, ferrocenyl), 6.7, 7.3, 7.7 (m, 6H, furanyl), 10.3 (s, 2H, N–NH)	15.2 (CH_3), 68.7, 70.5, 82.9 (ferrocenyl), 117.5, 121.8, 122.6, 131.5 (furanyl), 150.7 (C═N), 205.2 (C═O)
$\mathbf{L}^2$	2.2 (s, 6H, CH_3), 4.2–4.5 (m, 4H, ferrocenyl), 4.8–5.1 (m, 4H, ferrocenyl), 6.8, 7.4, 7.8 (m, 6H, thiophenyl), 10.4 (s, 2H, N–NH)	15.2 (CH_3), 68.7, 70.5, 82.9 (ferrocenyl), 118.4, 120.9, 122.8, 132.1 (thiophenyl), 150.7 (C═N), 205.2 (C═O)
$\mathbf{L}^3$	2.3 (s, 6H, CH_3), 4.2–4.5 (m, 4H, ferrocenyl), 4.8–5.1 (m, 4H, ferrocenyl), 7.2–7.4 (m, 2H, Ph) 7.5–7.6 (m, 2H, Ph), 7.7–7.9 (m, 4H, Ph), 10.3 (s, 2H, N–NH), 11.2 (s, 2H, OH)	15.0 (CH_3), 68.5, 70.4, 82.3 (ferrocenyl), 124.6, 122.4, 127.7, 138.6, 140.2, 175.8 (C–Ph), 151.7 (C═N), 205.3 (C═O)
10	2.3 (s, 6H, CH_3), 4.4–4.7 (m, 4H, ferrocenyl), 4.9–5.3 (m, 4H, ferrocenyl), 6.9, 7.5, 7.8 (m, 6H, furanyl), 10.5 (s, 2H, N–NH)	15.4 (CH_3), 68.9, 70.6, 83.1 (ferrocenyl), 117.6, 121.9, 122.8, 131.3 (furanyl), 150.9 (C═N), 205.4 (C═O)
11	2.3 (s, 6H, CH_3), 4.3–4.6 (m, 4H, ferrocenyl), 4.9–5.3 (m, 4H, ferrocenyl), 6.9, 7.6, 7.9 (m, 6H, thiophenyl), 10.5 (s, 2H, N–NH)	15.3 (CH_3), 68.8, 70.7, 82.9 (ferrocenyl), 118.5, 121.2, 123.1, 132.3 (thiophenyl), 150.9 (C═N), 205.5 (C═O)
12	2.5 (s, 6H, CH_3), 4.4–4.7 (m, 4H, ferrocenyl), 4.9–5.3 (m, 4H, ferrocenyl), 7.3–7.5 (m, 2H, Ph), 7.6–7.7 (m, 2H, Ph), 7.9–8.1 (m, 4H, Ph), 10.7 (s, 2H, N–NH), 11.6 (s, 2H, OH)	15.3 (CH_3), 68.7, 70.6, 82.5 (ferrocenyl), 124.9, 122.6, 127.8, 138.8, 140.3, 175.9 (C–Ph), 151.9 (C═N), 205.6 (C═O)

Table 3. Physical and analytical data of the metal(II) complexes **1–12**

No.	Metal complex, molecular formula	M.p. (°C)	BM (μ_{eff})	Analysis, calc. (found) (%) C	H	N	Yield (%)
1	$Co(L^1)Cl_2$ [615.7], $[C_{24}H_{22}FeCoCl_2N_4O_4]$	210–212	3.9	46.8 (46.5)	3.6 (3.9)	9.1 (9.5)	70
2	$Co(L^2)Cl_2$ [647.8], $[C_{24}H_{22}FeCoCl_2N_4O_2S_2]$	218–220	4.2	44.5 (44.9)	3.4 (3.2)	8.6 (8.4)	72
3	$Co(L^3)Cl_2$ [697.7], $[C_{28}H_{26}FeCoCl_2N_4O_4]$	215–217	4.1	54.5 (54.8)	3.7 (3.3)	8.0 (8.3)	70
4	$Cu(L^1)Cl_2$ [620.3], $[C_{24}H_{22}FeCuCl_2N_4O_4]$	222–224	1.3	46.4 (46.5)	3.5 (3.1)	9.0 (9.4)	68
5	$Cu(L^2)Cl_2$ [652.4], $[C_{24}H_{22}FeCuCl_2N_4O_2S_2]$	220–222	1.5	44.1 (44.6)	3.4 (3.7)	8.6 (8.2)	70
6	$Cu(L^3)Cl_2$ [702.3], $[C_{28}H_{26}FeCuCl_2N_4O_4]$	226–228	1.4	52.1 (52.6)	3.7 (3.5)	8.0 (8.3)	69
7	$Ni(L^1)Cl_2$ [615.7], $[C_{24}H_{22}FeNiCl_2N_4O_4]$	220–222	3.3	46.8 (46.6)	3.6 (3.9)	9.1 (9.5)	69
8	$Ni(L^2)Cl_2$ [647.8], $[C_{24}H_{22}FeNiCl_2N_4O_2S_2]$	225–227	3.4	44.5 (44.1)	3.4 (3.1)	8.6 (8.3)	68
9	$Ni(L^3)Cl_2$ [697.7], $[C_{28}H_{26}FeNiCl_2N_4O_4]$	218–220	3.5	52.5 (52.7)	3.7 (3.5)	8.0 (8.4)	70
10	$Zn(L^1)Cl_2$ [615.7], $[C_{24}H_{22}FeZnCl_2N_4O_4]$	226–228	–[a]	46.3 (46.2)	3.5 (3.1)	9.0 (9.3)	67
11	$Zn(L^2)Cl_2$ [647.8], $[C_{24}H_{22}FeZnCl_2N_4O_2S_2]$	220–222	–[a]	44.0 (44.4)	3.4 (3.5)	8.6 (8.4)	71
12	$Zn(L^3)Cl_2$ [697.7], $[C_{28}H_{26}FeZnCl_2N_4O_4]$	224–226	–[a]	52.0 (52.3)	3.7 (3.4)	8.0 (7.8)	70

[a] Diamagnetic.

NMR spectra

The ^{1}H and ^{13}C NMR spectral data of the free ligands and their diamagnetic zinc(II) chelates were obtained in DMSO-d_6. The spectral data are reported in Table 3, along with their possible assignments. All the protons were found in the expected region.[27] The conclusions drawn from these studies lend further support to the mode of bonding discussed in the IR spectra section. Furthermore, the IR and NMR spectra results suggest that the carbonyl group is coordinated with the metal ion through the keto form. In the spectra of diamagnetic zinc(II) complexes, these protons shifted downfield due to the increased conjugation and coordination to the metal atoms.[28] The number of protons calculated from the integration curves and those obtained from the values of the expected elemental analyses agree with each other. It was also observed that DMSO did not have any coordinating effect, either on the spectra of the ligands or on their metal complexes.

Electronic spectra

The cobalt(II) complexes exhibited well-resolved, low-energy bands at 7485–7670 cm^{-1} and 17 280–17 415 cm^{-1} and a strong high-energy band at 20 595–20 810 cm^{-1} (Table 4), which are assigned[29] to the transitions $^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(F)$, $^{4}T_{1g}(F) \rightarrow {}^{4}A_{2g}(F)$ and $^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(P)$ respectively for a high-spin octahedral geometry.[30] A high-intensity band at 28 115–28 360 cm^{-1} was assigned to the metal-to-ligand charge transfer. The magnetic susceptibility measurements (3.9–4.2 BM) for the solid cobalt(II) complexes are also

Table 4. Spectral data of the metal complexes **1–12**

Complex	IR (cm^{-1})	λ_{max} (cm^{-1})
1	1700 (C═O), 1590 (C═N), 485 (M–O), 435 (M–N), 315 (M–Cl)	7485, 17 280, 20 595, 28 115
2	1710 (C═O), 1590 (C═N), 485 (M–O), 435 (M–N), 315 (M–Cl)	7670, 17 415, 20 810, 28 360
3	1710 (C═O), 1590 (C═N), 485 (M–O), 435 (M–N), 315 (M–Cl)	7515, 17 335, 20 745, 28 275.
4	1700 (C═O), 1600 (C═N), 485 (M–O), 435 (M–N)	14 620, 19 160, 30 250
5	1710 (C═O), 1600 (C═N), 485 (M–O), 435 (M–N)	15 115, 19 375, 30 385
6	1710 (C═O), 1595 (C═N), 485 (M–O), 435 (M–N)	14 990, 19 265, 30 315
7	1710 (C═O), 1600 (C═N), 485 (M–O), 435 (M–N), 315 (M–Cl)	10 370, 15 485, 26 660, 29 735
8	1700 (C═O), 1590 (C═N), 485 (M–O), 435 (M–N), 315 (M–Cl)	10 415, 15 635, 26 810, 30 110
9	1710 (C═O), 1600 (C═N), 485 (M–O), 435 (M–N), 315 (M–Cl)	10 395, 15 540, 26 575, 29 925
10	1710 (C═O), 1595 (C═N), 485 (M–O), 435 (M–N), 315 (M–Cl)	28 350
11	1700 (C═O), 1595 (C═N), 485 (M–O), 435 (M–N), 315 (M–Cl)	29 285
12	1710 (C═O), 1600 (C═N), 485 (M–O), 435 (M–N), 315 (M–Cl)	28 775

indicative of three unpaired electrons per cobalt(II) ion, suggesting[31] consistency with their octahedral environment (Fig. 2a). The electronic spectra of the copper(II) complexes (Table 4) showed two low-energy weak bands at 14 620–15 115 cm^{-1} and 19 160–19 375 cm^{-1} and a high-energy strong band at 30 250–30 385 cm^{-1}; the two low-energy bands were assigned to the $^2B_{1g} \rightarrow {}^2A_{1g}$ transition and the high-energy band to the $^2B_{1g} \rightarrow {}^2E_g$ transition,[32] the latter being assigned to metal-to-ligand charge transfer. Also, the magnetic moment values (1.3–1.5 BM; Table 2) for the copper(II) complexes are indicative of antiferromagnetic spin–spin interaction through molecular association,[32] suggesting a square-planar geometry for the copper(II) complexes (Fig. 2b). The electronic spectra of the nickel(II) complexes showed d–d bands in the regions 10 370–10 415 cm^{-1}, 15 485–15 635 cm^{-1} and 26 660–26 810 cm^{-1}. These are assigned[33] 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 29 735–30 110 cm^{-1} was assigned to metal-to-ligand charge transfer. The magnetic measurements (3.3–3.5 BM) showed two unpaired electrons per nickel(II) ion, also suggesting[33] an octahedral geometry for the nickel(II) complexes (Fig. 2a). The electronic spectra of the zinc(II) complexes exhibited only a high-intensity band at 28 350–29 285 cm^{-1}, which is assigned[32] to a ligand-to-metal charge transfer.

Biological activity

Antibacterial activity

All compounds were tested against *E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, S. typhi, S. dysenteriae, B. cereus, C. diphtheriae, S. aureus* and *S. pyogenes* bacterial strains (Table 5) according to the literature protocol.[21] The results were compared with those of the standard drug imipenum. All ligands were found potentially active against one or more bacterial strains. The cobalt(II), copper(II), nickel(II) and zinc(II) metal complexes (**1–12**) of these synthesized ligands ($\mathbf{L}^1 - \mathbf{L}^3$) were also screened against the same bacterial strains. It was evident that the overall potency of the uncoordinated compounds/ligands was enhanced on coordination with the metal ions. However, potency of all the synthesized ligands and their metal complexes was lower than that of the standard drug, imipenum.

Antifungal activity

Antifungal screening of all compounds was carried out against *T. longifusus, C. albicans, Aspergillus flavus, M. canis, F. solani* and *C. glaberata* fungal strains according to the literature protocol.[21] The results were compared with the standard drugs miconazole and amphotericin B. These results, given in Table 6, indicate that all ligands were active against one or more fungal species; however, the metal(II) complexes (**1–12**) of these compounds showed enhanced activity compared with the uncoordinated compounds. However, the potency of all the synthesized ligands and their metal complexes was lower than that of the standard drugs, miconazole and amphotericin B.

MIC

The MIC of some selected compounds, which showed significant activity against selected bacterial species, was determined using the disc diffusion method.[21] The MIC of these compounds varies from 10 to 100 μg ml^{-1}. The results, as shown in Table 7, indicate that these compounds are most active by inhibiting the growth of the organisms tested at 10 μg ml^{-1} concentrations.

The biological activity data exhibited a marked enhancement on coordination with the metal ions against all the test bacterial/fungal strains. The compounds generally showed moderate antibacterial activity against two or four species and insignificant activity against one or two species. However, they showed good 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 may be rationalized

Table 5. *In vitro* antibacterial activity data of the ligands $\mathbf{L^1}$–$\mathbf{L^3}$ and metal(II) complexes **1–12**

	Diameter of zones showing complete inhibition of growth[a] (mm)									
Compound	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*	*S. typhi*	*S. dysenteriae*	*B. cereus*	*C. diphtheriae*	*S. pyogenes*	*K. pneumoniae*
$\mathbf{L^1}$	14	12	7	16	12	15	6	5	12	12
$\mathbf{L^2}$	16	14	10	17	16	18	8	10	12	13
$\mathbf{L^3}$	16	12	16	18	18	15	12	13	14	15
1 $Co(L^1)Cl_2$	18	15	21	20	21	22	18	15	15	19
2 $Co(L^2)Cl_2$	18	16	20	22	22	21	16	16	17	20
3 $Co(L^3)Cl_2$	20	15	21	20	19	21	19	17	18	20
4 $Cu(L^1)Cl_2$	21	11	22	20	20	22	16	14	18	21
5 $Cu(L^2)Cl_2$	21	16	20	22	20	22	18	18	19	21
6 $Cu(L^3)Cl_2$	20	18	20	21	22	20	16	15	20	20
7 $Ni(L^1)Cl_2$	21	20	22	20	21	22	15	15	18	19
8 $Ni(L^2)Cl_2$	20	15	22	23	18	22	18	16	19	18
9 $Ni(L^3)Cl_2$	18	16	22	18	22	22	15	15	18	18
10 $Zn(L^1)Cl_2$	18	14	22	20	21	21	18	14	20	20
11 $Zn(L^2)Cl_2$	20	15	21	22	20	22	16	15	17	19
12 $Zn(L^3)Cl_2$	21	16	19	18	21	20	15	15	18	20
Imipenum[b]	30	30	25	30	32	30	30	25	30	32

[a] Ligand: >15 mm, significant activity; 7–14 mm, moderate activity; <7 mm, weak activity.
[b] Standard drug.

Table 6. *In vitro* antifungal activity data of the ligands $\mathbf{L^1}$–$\mathbf{L^3}$ and metal(II) complexes **1–12**

	Diameter of zones showing complete inhibition of growth[a] (mm)					
Compound	*T. longifusus*	*C. albicans*	*A. flavus*	*M. canis*	*F. solani*	*C. glaberata*
$\mathbf{L^1}$	16	11	10	16	12	11
$\mathbf{L^2}$	15	12	12	14	15	14
$\mathbf{L^3}$	16	13	12	15	10	15
1	18	15	15	18	18	16
2	18	12	15	18	17	18
3	20	24	15	16	18	16
4	18	12	14	18	18	18
5	21	11	15	16	06	17
6	20	15	14	18	16	18
7	20	15	16	17	18	18
8	18	16	16	18	16	16
9	20	15	18	17	15	18
10	18	16	15	18	18	15
11	20	15	15	17	17	16
12	18	14	18	18	18	17
Miconazole[b]	30	–	25	25	–	25
Amphotericin B[b]	–	25	30	–	30	30

[a] Ligand: > 14 mm, significant activity; 7–13 mm, moderate activity; <7 mm, weak activity. Dashes indicate not tested.
[b] Standard drug.

on the basis that their structures possess an additional C═N bond. It has been suggested that ligands with nitrogen and oxygen donor systems inhibit enzyme activity, since the enzymes that require these groups for their activity appear to be more susceptible to deactivation by the metal ions on coordination. Moreover, coordination reduces the polarity[34,35] of the metal ion mainly because of the partial sharing of its positive charge with the

Table 7. MIC of selected compounds against selected bacterial species[a]

Compound	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*	*S. typhi*	*S. dysenteriae*	*B. cereus*	*C. diphtheriae*	*S. pyogenes*	*S. aureus*
L[1]	10	–	–	>100	–	–	–	–	–	–
L[2]	10	10	–	>100	10	10	–	–	–	–
L[3]	–	–	10	10	>100	–	–	–	–	–
1	10	10	>100	25	10	10	10	–	>100	25
2	10	25	10	–	>100	–	>100	>100	10	10
3	10	>100	>100	>100	>100	>100	>100	>100	10	–
4	–	–	10	–	10	10	–	–	25	10
5	10	>100	>100	10	10	–	10	–	10	10
6	>100	>100	25	25	–	10	–	–	>100	>100
7	10	10	>100	25	>100	>100	–	10	>100	–
8	–	>100	>100	10	>100	10	>100	10	>100	10
9	10	–	>100	>100	>100	>100	>100	10	–	>100
10	>100	>100	10	10	>100	10	>100	–	>100	>100
11	25	10	>100	10	–	–	–	>100	10	>100
12	>100	–	10	>100	25	>100	–	–	10	>100
Imipenum[b]	10	10	25	10	10	10	25	10	10	10

[a] Dashes indicate not tested.
[b] Standard drug.

donor groups[36–39] within the chelate ring system formed during coordination. This process, in turn, increases the lipophilic nature of the central metal atom, which favours its permeation more efficiently through the lipoid layer of the micro-organism,[40–43] thereby destroying them more aggressively.

REFERENCES

1. Campbell MJM. *Coord. Chem. Rev.* 1975; **15**: 279.
2. Williams DR. *Chem. Rev.* 1972; **72**: 203.
3. Furst A, Haro RA. *Prog. Exp. Tumor Res.* 1969; **12**: 102.
4. Raizada MS, Srinisvastava MN. *Synth. React. Inorg. Met. Org. Chem.* 1993; **22**: 393.
5. West DS, Liberta AE, Padhye SB, Chikate PB, Sonowane AS. *Coord. Chem. Rev.* 1993; **123**: 49.
6. Fang C-J, Duan C-Y, He C, Meng Q-J. *Chem. Commun.* 2000; 1187.
7. Padhy S, Kaufman GB. *Coord. Chem. Rev.* 1985; **63**: 127.
8. Bauer DJ, St Vincent L, Kempe CH, Downe AW. *Lancet* 1963; **20**: 494.
9. Petering HG, Buskik HH, Underwood GE. *Cancer Res.* 1964; **64**: 367.
10. Johnson CW, Jolyner JW, Perry RP. *Antibiot. Chemother.* 1952; **2**: 636.
11. Ming LJ. *Med. Res. Rev.* 2003; **23**: 697.
12. Klayman DL, Scovil JP, Bartosevich JF, Bruce J. *J. Med. Chem.* 1983; **26**: 35.
13. Casas JS, Garcia-Tasende MS, Sordo J. *Coord. Chem. Rev.* 2000; **209**: 197.
14. Longato B, Pilloni G, Valle G, Gorain B. *Inorg. Chem.* 1988; **27**: 956.
15. Hill DT, Girard GR, McCabe EL, Johnson RK, Stupik PD, Zhang JH, Reiff, WM, Eggeieston DS. *Inorg. Chem.* 1998; **28**: 3529.
16. Edwards EI, Epton R, Marr G. *J. Organometal. Chem.* 1975; **85**: C–23.
17. Chohan ZH, Praveen M. *Appl. Organometal. Chem.* 2000; **14**: 376.
18. Chohan ZH. *Appl. Organometal. Chem.* 2002; **16**: 17.
19. Chohan ZH, Praveen M. *Synth. React. Inorg. Met. Inorg. Chem.* 2000; **30**: 175.
20. Chohan ZH, Scozzafava, A, Supuran CT. *Synth. React. Inorg. Met. Org. Chem.* 2003; **33**: 241.
21. Atta-ur-Rahman, Choudhary MI, Thomsen WJ. *Bioassay Techniques for Drug Development*. Harwood Academic Publishers: The Netherlands, 2001; 16.
22. Geary WJ. *Coord. Chem. Rev.* 2001; **7**: 81.
23. Nakamoto K. *Infrared Spectra of Inorganic and Coordination Compounds*, 2nd edn. Wiley Interscience: New York, 1970.
24. Agarwal RK. *J. Indian Chem. Soc.* 1988; **65**: 448.
25. Bellamy LJ. *The Infrared Spectra of Complex Molecules*. Wiley: New York, 1971.
26. Ferrero JR. *Low-Frequency Vibrations of Inorganic and Coordination Compounds*. Wiley: New York, 1971.
27. Simmons WW. *The Sadtler Handbook of Proton NMR Spectra*. Sadtler Research Laboratories, Inc.: 1978.
28. Pasto DJ. *Organic Structure Determination*. Prentice Hall International: 1969.
29. Lever ABP, Lewis J. *J. Chem. Soc.* 1963; 2552.
30. Carlin RL. *Transition Metal Chemistry*, 2nd edn. Marcel Dekker: New York, 1965.
31. Estes WE, Govel DP, Halfield WB, Hodgson DJ. *Inorg. Chem.* 1978; **17**: 1415.
32. Balhausen CJ. *An Introduction to Ligand Field*. McGraw Hill: New York, 1962.
33. Lever ABP. *Inorganic Electronic Spectroscopy*. Elsevier: Amsterdam, 1984.
34. Chohan ZH, Munawar A, Supuran CT. *Metal-Based Drugs* 2001; **8**: 137.
35. Chohan ZH, Supuran CT. *Main Group Met. Chem.* 2001; **24**: 399.
36. Hassan MU, Chohan ZH, Supuran CT. *Main Group Met. Chem.* 2002; **25**: 291.
37. Chohan ZH, Scozzafava A, Supuran CT. *J. Enzym. Inhib. Med. Chem.* 2003; **17**: 261.
38. Chohan ZH, Farooq MA, Scozzafava A, Supuran CT. *J. Enzym. Inhib. Med. Chem.* 2002; **17**: 1.

39. Chohan ZH, Iqbal MS, Iqbal HS, Scozzafava A, Supuran CT. *J. Enzym. Inhib. Med. Chem.* 2002; **17**: 87.
40. Chohan ZH, Scozzafava A, Supuran CT. *J. Enzym. Inhib. Med. Chem.* 2003; **18**: 259.
41. Chohan ZH, Supuran CT, Scozzafava A. *J. Enzym. Inhib. Med. Chem.* 2004; **19**: 79.
42. Chohan ZH. *Synth. React. Inorg. Met. Org. Chem.* 2004; **34**: 833.
43. Hassan MU, Chohan ZH, Scozzafava A, Supuran CT. *J. Enzym. Inhib. Med. Chem.* 2004; **19**: 263.