Journal of Enzyme Inhibition and Medicinal Chemistry, October 2004 Vol. 19 (5), pp. 417–423

Isatin-derived Antibacterial and Antifungal Compounds and their Transition Metal Complexes

ZAHID H. CHOHAN[a], HUMAYUN PERVEZ[a], A. RAUF[b], KHALID M. KHAN[c] and CLAUDIU T. SUPURAN[d,*]

[a]*Department of Chemistry, Bahauddin Zakariya University, Multan, Pakistan;* [b]*Department of Chemistry, Islamia University, Bahawalpur, Pakistan;* [c]*HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi, Pakistan;* [d]*Dipartmento di Chimica, Laboratorio di Chimica Bioinorganica, University of Florence, Via della Lastruccia 3, Rm. 188, Polo Scientifico, 50019, Sesto Fiorentino, Firenze, Italy*

(Received 6 October 2003; In final form 15 March 2004)

A series of isatins incorporating thiazole, thiadiazole, benzothiazole and *p*-toluene sulfonyl hydrazide moieties, along with their cobalt(II), copper(II), nickel(II) and zinc(II) metal complexes have been synthesized and characterized by elemental analyses, molar conductances, magnetic moments, IR, NMR and electronic spectral data. These compounds have been screened for antibacterial activity against *Escherichia coli*, *Bacillus subtillis*, *Shigella flexneri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*, and for antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberata* using the agar-well diffusion method. All the synthesized compounds have shown good affinity as antibacterial and/or antifungal agents which increased in most of the cases on complexation with the metal ions.

Keywords: Isatins; Metal complexes; Antibacterial; Antifungal

INTRODUCTION

The chemical versatility of isatin (2,3-indolinone) derivatives has led to their extensive use as synthons for the preparation of many biologically active compounds.[1–6] Hydrazine-derived isatins were found to be active against carcino-sarcoma 256.[6,7] Similarly, acetone- and ketone-derived isatins exhibited anticonvulsant activity.[8] Another class of thiosemicarbazone-derived isatins was found to exhibit interesting applications as research tools in physiological studies.[9–13] Similarly, many other isatin-derived compounds possess a wide spectrum of medicinal properties and thus, have been studied for activity against tuberculosis,[14,15] leprosy,[16] fungal,[17–20] viral[21] and bacterial[13,22] infections, rheumatism,[23] trypanosomiasis[24] and as anticonvulsants.[25–28] Because of such significant biological activities, we thought it worthwhile to incorporate the chemistry of isatin with thiazole, thiadiazole, benzothiazole and *p*-toluene sulfonyl hydrazide and to prepare novel ligands ($\mathbf{L}^1$–$\mathbf{L}^4$) (Figure 1), and their cobalt(II), copper(II), nickel(II) and zinc(II) complexes. It is expected that such compounds would carry medicinal properties thus introducing another class of isatins incorporating metal-based antibacterials and antifungals. The synthesized ligands and their metal complexes have been characterized by IR, NMR, UV-Visible, molar conductance, magnetic moment and elemental analyses data. All the ligands, along with their metal complexes were screened for antibacterial activity against *Escherichia coli*, *Bacillus subtillis*, *Shigella flexneri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* and for antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberata* respectively, using the agar-well diffusion method. Miconazole and ketoconazole were used as reference antifungal drugs. The ligands showed varied antibacterial and antifungal activity against two or more strains and their activity was enhanced respectively on coordination/chelation.

MATERIAL AND METHODS

Solvents used were analytical grade; all metals (II) were used as chloride salts. IR spectra were recorded

*Corresponding author. Tel.: +39-055-457-3005. Fax: +39-055-457-3385. E-mail: claudiu.supuran@unifi.it

ISSN 1475-6366 print/ISSN 1475-6374 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/14756360410001710383

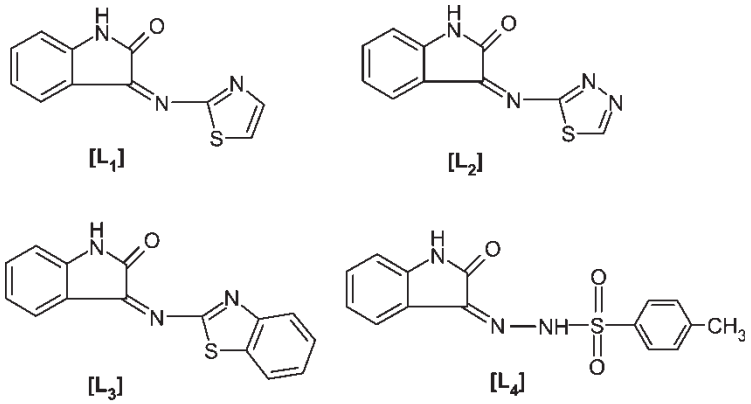

FIGURE 1 Proposed structure of the ligands ($\mathbf{L}^1$–$\mathbf{L}^4$).

on the Philips Analytical PU 9800 FTIR spectrophotometer. NMR spectra were recorded 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 Gouy's method. Melting points were recorded on a Gallenkamp (U.K.) apparatus and are not corrected. The complexes were analyzed for their metal contents by EDTA titration.[29] Antibacterial and antifungal screening was done at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Preparation of Ligands ($\mathbf{L}^1$–$\mathbf{L}^4$) and Metal (II) Complexes (1–16)

To a stirred solution of 2-aminothiazole (3.0 g, 0.03 mol) in warm ethanol (50 mL) was added isatin (4.4 g, 0.03 mol) in ethanol (60 mL). Then 2–3 drops of conc. H_2SO_4 were added and the mixture refluxed for 3 h. The completion of reaction was monitored by TLC. After completion of 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 $\mathbf{L}^1$ (Figure 1). The same method was used for the preparation of $\mathbf{L}^2$–$\mathbf{L}^4$ from the corresponding heteroaromatic/aromatic amines, working under the same conditions with the same respective molar ratio.

For the preparation of metal (II) complexes, a solution (20 mL) of the corresponding ligand (0.02 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 when it solidified on cooling. The solid obtained was filtered, washed with ethanol, then with ether and dried in air. Crystallization from aqueous/ethanol (30:70) gave the desired metal complex. The same method was used for the preparation of all other complexes by using their respective metal (II) salts.

Biological Activity

All the synthesized ligands ($\mathbf{L}^1$–$\mathbf{L}^4$) and their corresponding metal(II) complexes (**1–16**) were screened *in-vitro* for their antibacterial activity against *Escherichia coli*, *Bacillus subtillis*, *Shigella flexneri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* and for antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberata* using the agar well diffusion method.[30,31] Two to eight hours old bacterial/fungal 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. The recommended concentration (100 μl) of the test sample (1 mg/ml in DMSO) was introduced into the corresponding wells. Other wells supplemented with DMSO and reference antibacterial drugs 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). Imipenum was used as a standard drug for antibacterial activity and, Miconazole and Amphotericin B for antifungal activity.

RESULTS AND DISCUSSION

Chemistry

The ligands ($\mathbf{L}^1$–$\mathbf{L}^4$) were prepared by refluxing the appropriate amount of an ethanolic solution of isatin with the corresponding heteroaromatic/aromatic amines, in 1:1 molar ratio. The structures of these

TABLE I Physical, spectral and analytical data of the ligands $\mathbf{L}^1$–$\mathbf{L}^4$

Ligand/Mol. form	M.P (°C)	IR (cm^{-1})	C, H, N; Calc. (found) %	Yield (%)
$\mathbf{L}^1$ [229.0] ($C_{11}H_7N_3OS$)	136	3315 (NH), 1715 (C═O), 1645 (C═N), 1560 (C═N)	57.6 (57.9), 3.1 (3.5), 18.3 (18.0)	62
$\mathbf{L}^2$ [230.0] ($C_{10}H_6N_4OS$)	148	3315 (NH), 1715 (C═O), 1655 (C═N), 1560 (C═N), 1425 (N—N)	52.2 (52.6), 2.6 (2.2), 24.3 (24.7)	60
$\mathbf{L}^3$ [279.0] ($C_{15}H_9N_3OS$)	165	3315 (NH), 1715 (C═O), 1650 (C═N), 1575 (C═N)	64.5 (64.8), 3.2 (3.6), 15.1 (15.0)	65
$\mathbf{L}^4$ [315.0] ($C_{15}H_{13}N_3O_3S$)	188	3315 (NH), 1715 (C═O), 1655 (C═N), 1415 (N—NH), 1350, 1155 (S═O).	57.1 (57.5), 4.1 (3.8), 13.3 (13.7)	63

synthesized ligands were established with the aid of their IR, NMR and microanalytical data (Tables I, II). All metal complexes (**1–16**) (Table III) of these ligands were prepared by the stoichiometric reaction of the corresponding ligand with the respective metal salt as chloride in a molar ratio M:L of 1:2. They are all air and moisture stable and, are intensely colored amorphous solids, which decompose without melting. They are insoluble in common organic solvents and only soluble in water, DMF and DMSO. Molar conductance values of the soluble complexes in DMF (1 mM solution at 25°C), indicated high values (88–97 $ohm^{-1}cm^{-2}mol^{-1}$) suggesting that they are all electrolytic in nature.[32]

The elemental analyses data agree well with the proposed formulae for the ligands and also confirmed the $[M(L)_2]Cl_2$ composition for the complexes with ligands, $\mathbf{L}^1$, $\mathbf{L}^2$ and $\mathbf{L}^3$, and $[M(L)_2Cl_2]$ composition for the metal (II) complexes with $\mathbf{L}^4$. Efforts to grow good crystals of the ligands and their metal complexes for X-ray diffraction studies were unsuccessful due to their poor solubility in common organic solvents.

IR Spectra

The selected IR spectra of the ligands and their metal complexes along with their tentative assignments are reported in Tables I and IV. The IR spectra of the complexes (Table IV) showed a lower shift of wave numbers in ν(C═N) of both the azomethine and heteroaromatic moieties by 15–20 cm^{-1}, respectively. The band located at 1715 cm^{-1} in all the ligands attributed[33] to ν (C═O) moiety of the isatin also moved to the lower frequency side by 15–20 cm^{-1} on coordination. This data on comparison with the spectra of the chelates suggests that the azomethine-N, heteroaromatic-N and isatin-O of the ligands, $\mathbf{L}^1$, $\mathbf{L}^2$ and $\mathbf{L}^3$ are involved in coordination with the metal ions. The copper complexes of these ligands, however, showed only the involvement of bonding of azomethine-N and

TABLE II ^{1}H NMR and ^{13}C NMR data of the ligands ($\mathbf{L}^1$–$\mathbf{L}^4$) and Zn (II) complexes

No.	^{1}H NMR (DMSO-d_6)(ppm)	^{13}C NMR (DMSO-d_6)(ppm)
$\mathbf{L}^1$	10.2 (s, 1H, NH), 7.1–7.4 (m, 4H, isatin-Ph), 7.6 (dd, 1H, thiazol), 7.8 (dd, 1H, thiazol).	210.6 (C═O), 152.5 (C═N), 149.5, 140.6, 125.4, 124.3, 120.5, 110.8 (isatin-Ph), 153.8, 142.5, 119.7 (thiazol).
$\mathbf{L}^2$	10.2 (s, 1H, NH), 7.2–7.5 (m, 4H, isatin-Ph), 8.1 (s, 1H, thiadiazol).	210.7 (C═O), 152.8 (C═N), 149.6, 140.6, 125.4, 124.4, 120.5, 110.8 (isatin-Ph), 154.2, 152.5, (thiadiazol).
$\mathbf{L}^3$	10.2 (s, 1H, NH), 7.1–7.5 (m, 4H, isatin-Ph), 7.3–8.2 (m, 4H, benzothiazol).	210.6 (C═O), 152.4 (C═N), 149.5, 140.5, 125.5, 124.3, 120.6, 110.8 (isatin-Ph), 152.4, 151.2, 150.3, 125.6, 124.4, 120.4., 110.9 (benzothiazol).
$\mathbf{L}^4$	2.6 (s, 3H, CH_3), 10.2 (s, 1H, NH), 11.1 (s, 1H, N—NH), 7.1–7.4 (m, 4H, isatin-Ph), 7.3–7.8 (m, 4H, toluene).	53.7 (CH_3), 210.7 (C═O), 152.6 (C═N), 149.6, 140.5, 125.5, 124.4, 120.6, 110.8 (isatin-Ph), 128.4, 127.6, 120.5, 113.4, (toluene).
13	10.2 (s, 1H, NH), 7.2–7.5 (m, 4H, isatin-Ph), 7.7 (dd, 1H, thiazol), 7.9 (dd, 1H, thiazol).	210.8 (C═O), 152.7 (C═N), 149.5, 140.7, 125.5, 124.3, 120.6, 110.8 (isatin-Ph), 153.8, 142.7, 119.8 (thiazol).
14	10.2 (s, 1H, NH), 7.3–7.6 (m, 4H, isatin-Ph), 8.3 (s, 1H, thiadiazol).	210.8 (C═O), 152.9 (C═N), 149.7, 140.6, 125.5, 124.4, 120.6, 110.8 (isatin-Ph), 154.4, 152.6, (thiadiazol).
15	10.2 (s, 1H, NH), 7.2–7.5 (m, 4H, isatin-Ph), 7.4–8.4 (m, 4H, benzothiazol).	210.7 (C═O), 152.6 (C═N), 149.5, 140.6, 125.5, 124.4, 120.7, 110.8 (isatin-Ph), 152.5, 151.2, 150.4, 125.6, 124.6, 120.5, 110.9 (benzothiazol).
16	2.6 (s, 3H, CH_3), 10.2 (s, 1H, NH), 11.3 (s, 1H, N—NH), 7.2–7.5 (m, 4H, isatin-Ph), 7.4–7.8 (m, 4H, toluene).	53.7 (CH_3), 210.9 (C═O), 152.9 (C═N), 149.7, 140.5, 125.5, 124.5, 120.7, 110.8 (isatin-Ph), 128.5, 127.6, 120.6, 113.6, (toluene).

TABLE III Physical and analytical data of the metal (II) complexes **1–16**

No.	Metal complex/ Mol. formula	M.P (°C)	B.M (μ_{eff})	C, H, N; Calc. (found) %	Yield (%)
1	$[Co(L^1)_2]Cl_2$ [587.9] ($C_{22}H_{14}N_6CoCl_2O_2S_2$)	224–226	3.8	44.9 (44.5), 2.4 (2.7), 14.3 (14.6)	68
2	$[Co(L^2)_2]Cl_2$ [589.9] ($C_{20}H_{12}N_8CoCl_2O_2S_2$)	222–224	4.1	40.7 (40.3), 2.0 (2.2), 19.0 (19.4)	70
3	$[Co(L^3)_2]Cl_2$ [687.9] ($C_{30}H_{18}N_6CoCl_2O_2S_2$)	196–198	3.9	52.3 (52.8), 2.6 (2.3), 12.2 (12.4)	72
4	$[Co(L^4)_2]Cl_2$ [759.9] ($C_{30}H_{26}N_6CoCl_2O_6S_2$)	226–228	4.1	47.4 (47.6), 3.4 (3.1), 11.1 (11.3)	69
5	$[Cu(L^1)_2]Cl_2$ [592.5] ($C_{22}H_{14}N_6CuCl_2O_2S_2$)	222–224	1.3	44.6 (44.9), 2.4 (2.1), 14.2 (14.0)	68
6	$[Cu(L^2)_2]Cl_2$ [594.5] ($C_{20}H_{12}N_8CuCl_2O_2S_2$)	226–228	1.6	40.4 (40.9), 2.0 (2.4), 18.8 (18.5)	70
7	$[Cu(L^3)_2]Cl_2$ [692.5] ($C_{30}H_{18}N_6CuCl_2O_2S_2$)	228–230	1.4	52.0 (52.2), 2.6 (2.5), 12.1 (12.4)	65
8	$[Cu(L^4)_2]Cl_2$ [764.5] ($C_{30}H_{26}N_6CuCl_2O_6S_2$)	218–220	1.6	47.1 (47.3), 3.4 (3.5), 11.0 (11.3)	71
9	$[Ni(L^1)_2]Cl_2$ [587.7] ($C_{22}H_{14}N_6NiCl_2O_2S_2$)	220–222	3.1	44.9 (44.6), 2.4 (2.7), 14.3 (14.5)	69
10	$[Ni(L^2)_2]Cl_2$ [589.7] ($C_{20}H_{12}N_8NiCl_2O_2S_2$)	225–227	3.3	40.7 (40.5), 2.0 (2.2), 19.0 (19.3)	67
11	$[Ni(L^3)_2]Cl_2$ [687.7] ($C_{30}H_{18}N_6NiCl_2O_2S_2$)	227–229	3.2	52.3 (52.6), 2.6 (2.2), 12.2 (12.7)	70
12	$[Ni(L^4)_2]Cl_2$ [759.7] ($C_{30}H_{26}N_6NiCl_2O_6S_2$)	223–225	3.3	47.4 (47.0), 3.4 (3.7), 11.1 (11.4)	68
13	$[Zn(L^1)_2]Cl_2$ [594.4] ($C_{22}H_{14}N_6ZnCl_2O_2S_2$)	226–228	Dia	44.4 (44.7), 2.4 (2.0), 14.1 (14.3)	67
14	$[Zn(L^2)_2]Cl_2$ [596.4] ($C_{20}H_{12}N_8ZnCl_2O_2S_2$)	220–222	Dia	40.2 (40.5), 2.0 (2.4), 18.8 (18.4)	71
15	$[Zn(L^3)_2]Cl_2$ [694.4] ($C_{30}H_{18}N_6ZnCl_2O_2S_2$)	230–232	Dia	51.8 (51.4), 2.6 (2.8), 12.1 (12.4)	67
16	$[Zn(L^4)_2]Cl_2$ [766.4] ($C_{30}H_{26}N_6ZnCl_2O_6S_2$)	228–230	Dia	47.0 (47.4), 3.4 (3.9), 11.0 (11.3)	68

isatin-O. IR spectra of the cobalt, nickel and zinc complexes with $\mathbf{L}^4$ showed bands assigned to the azomethine-N and isatin-O and a new band at 315 cm^{-1}, suggesting[34] coordination of ν(M—Cl) in $[M(L)_2Cl_2]$. The far IR spectra of the metal complexes exhibited new bands which are not present in the spectra of the ligands. These bands are located at 428, 435 and 415 cm^{-1}, assigned[35,36] to ν(M—N) of azomethine-N, ν (M—N) of heteroaromatic-N and ν (M═O) of isatin-O, supporting evidence for the bonding of the ligands with the metal ions. Accordingly, the above mentioned data suggest that the ligands, $\mathbf{L}^1$, $\mathbf{L}^2$ and $\mathbf{L}^3$, behave as tridentate towards cobalt (II), nickel (II) and zinc (II) complexes and bidentate towards copper complexes whereas $\mathbf{L}^4$, behaved as bidentate towards cobalt (II), copper (II), nickel (II) and zinc (II) complexes. Thus, the cobalt (II), nickel (II), copper (II) and zinc (II) complexes with $\mathbf{L}^4$, showed octahedral geometry via the coordination through azomethine-N, isatin-O and the coordination of a chloride atom. The bands present in the spectra of $\mathbf{L}^4$ at 1350 and 1155 cm^{-1} assigned to ν $(O═S═O)_{asymmet,symmet}$ however, remained unchanged in the spectra of its metal complexes indicating, non-participation of this group.

NMR Spectra

The ^1H NMR and ^{13}C NMR spectra of the free ligands and their diamagnetic Zn (II) chelates (**13–16**) were run in DMSO-d_6. The ^1H NMR spectral data are reported along with the possible assignments in

TABLE IV Spectral data of the metal complexes **1–16**

No.	IR (cm^{-1})	λ_{max} (cm^{-1})
1	3315 (NH), 1700 (C═O), 1625 (C═N), 1540 (C═N), 435 (M—N), 428 (M—N), 415 (M—O),	7,380, 17,265, 20,550, 29,210.
2	3315 (NH), 1700 (C═O), 1625 (C═N), 1545 (C═N), 435 (M—N), 428 (M—N), 415 (M—O).	7,475, 17,380, 20,675, 29,365
3	3315 (NH), 1695 (C═O), 1630 (C═N), 1545 (C═N), 435 (M—N), 428 (M—N), 415 (M—O).	7,395, 17,280, 20,585, 29,275.
4	3315 (NH), 1700 (C═O), 1625 (C═N), 1540 (C═N), 1350, 1155 (S═O), 435 (M—N), 415 (M—O).	7,455, 17,315, 20,625, 29,190.
5	3315 (NH), 1700 (C═O), 1625 (C═N), 435 (M—N), 415 (M—O).	15,115 19,460, 30,135
6	3315 (NH), 1695 (C═O), 1625 (C═N), 435 (M—N), 415 (M—O).	15,255, 19,615, 30,310
7	3315 (NH), 1700 (C═O), 1630 (C═N), 435 (M—N), 415 (M—O).	15,170, 19,595, 30,245
8	3315 (NH), 1695 (C═O), 1630 (C═N), 1350, 1155 (S═O), 435 (M—N), 415 (M—O),315 (M—Cl).	15,235, 19,540, 30,275
9	3315 (NH), 1700 (C═O), 1625 (C═N), 1545 (C═N), 435 (M—N), 428 (M—N), 415 (M—O).	10,280, 15,615, 26,360, 29,905.
10	3315 (NH), 1700 (C═O), 1625 (C═N), 1540 (C═N), 435 (M—N), 428 (M—N), 415 (M—O).	10,315, 15,585, 26,415, 30,215.
11	3315 (NH), 1700 (C═O), 1630 (C═N), 1545 (C═N), 435 (M—N), 428 (M—N), 415 (M—O).	10,295, 15,710, 26,395, 30,145.
12	3315 (NH), 1695 (C═O), 1625 (C═N), 1545 (C═N), 1350, 1155 (S═O), 435 (M—N), 415 (M—O), 315 (M—Cl).	10,310, 15,615, 26,380, 30,185.
13	3315 (NH), 1700 (C═O), 1625 (C═N), 435 (M—N), 415 (M—O).	28,345.
14	3315 (NH), 1700 (C═O), 1625 (C═N), 435 (M—N), 415 (M—O).	29,180.
15	3315 (NH), 1695 (C═O), 1630 (C═N), 435 (M—N), 415 (M—O).	28,780.
16	3315 (NH), 1695 (C═O), 1630 (C═N), 1350, 1155 (S═O), 435 (M—N), 415 (M—O), 315 (M—Cl).	28,905.

Table II. All the protons due to heteroaromatic/aromatic groups were found to be in their expected region.[37] The conclusions drawn from these studies lend further support to the mode of bonding discussed in their IR spectra. In the spectra of diamagnetic Zn (II) complexes (**13**–**16**) these protons shifted downfield due to the increased conjugation and coordination to the metal atoms.[38] The number of protons calculated from the integration curves, and those obtained from the values of the expected CHN analyses agree with each other. In the ^{13}C NMR spectra, the ligands and their Zn (II) complexes display signals assigned respectively to heteroaromatic/aromatic carbons. These signals also appear downfield in comparison with the corresponding signals of the ligands indicating[38] coordination with the central metal atom. It was observed that DMSO did not have any coordinating effect on the spectra of the ligands or their metal complexes.

Electronic Spectra

The Co(II) complexes exhibited well-resolved, low-energy bands at 7,380–7,475 cm^{-1}, 17,265–17,380 cm^{-1} and a strong high-energy band at 20,550–20,675 cm^{-1} (Table IV) which are assigned[39] 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.[40] A high intensity band at 21,190–29,365 cm^{-1} was assigned to the metal to ligand charge transfer. The magnetic susceptibility measurements (3.8–4.1 BM) for the solid Co (II) complexes are also indicative of three unpaired electrons per Co (II) ion suggesting[41] consistency with their octahedral environment.

The electronic spectra of the Cu (II) complexes (Table IV) showed two low-energy weak bands at 15,115–15,255 cm^{-1} and 19,460–19,615 cm^{-1} and a strong high-energy band at 30,135–30,310 cm^{-1} and may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively.[42] The strong high-energy band, in turn, is assigned to metal $\rightarrow$ ligand charge transfer. Also, the magnetic moment values (1.3–1.6 BM) (Table III) for the copper (II) complex are indicative of anti-ferromagnetic spin–spin interaction through molecular association.[42]

The electronic spectra of the Ni (II) complexes showed d-d bands in the region 10,280–10,315, 15,585–15,710 and 26,360–26,415 cm^{-1}. These are assigned[43] to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T1_g(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$, respectively, consistent with their well-defined octahedral configuration. The band at 29,905–30,215 cm^{-1} was assigned to metal $\rightarrow$ ligand charge transfer. The magnetic measurements (3.1–3.3 BM) showed two unpaired electrons per Ni (II) ion suggesting[42] also an octahedral geometry for the Ni (II) complexes. The electronic spectra of the Zn (II) complexes exhibited only a high-intensity band at 28,345–29,180 cm^{-1} which is assigned[43] to a ligand-metal charge transfer.

BIOLOGICAL ACTIVITY

The antibacterial and antifungal activity results presented in Tables V and VI, show clearly that all

TABLE V Antibacterial activity of compounds $\mathbf{L}^1$–$\mathbf{L}^4$ and **1**–**16**

Diameter of zones showing complete inhibition of growth (mm)*						
Compound	*E. coli*	*P. aeruginosa*	*B. subtillis,*	*S. flexneri*	*S. aureus*	*S. typhi*
$\mathbf{L}^1$	8	12	9	10	9	10
$\mathbf{L}^2$	9	11	10	11	7	11
$\mathbf{L}^3$	10	13	7	12	8	10
$\mathbf{L}^4$	9	12	8	13	10	10
1	10	15	10	14	12	12
2	12	16	12	15	10	11
3	13	14	11	16	13	12
4	14	15	12	15	10	13
5	14	14	12	14	12	14
6	15	14	11	17	12	12
7	16	14	11	18	10	12
8	15	16	13	15	10	11
9	12	17	14	17	12	12
10	15	12	12	16	13	12
11	12	14	13	13	10	13
12	12	12	14	17	13	13
13	13	13	15	18	12	11
14	14	14	14	17	11	12
15	15	15	13	15	12	13
16	14	16	13	13	12	11
Impenan	15	18	15	15	14	15

* $>$ 14 mm = significant activity; 7–13 mm = moderate activity; $<$ 7 mm = weak activity.[30,31]

TABLE VI Antifungal activity of compounds $\mathbf{L}^1$–$\mathbf{L}^4$ and **1**–**16**

Compound	Diameter of zones showing complete inhibition of growth (mm)* *T. longifusus*	*C. albicans*	*A. flavus*	*M. canis*	*F. solani*	*C. glaberata*
$\mathbf{L}^1$	18	12	18	22	20	20
$\mathbf{L}^2$	15	12	20	20	20	15
$\mathbf{L}^3$	16	11	20	21	18	15
$\mathbf{L}^4$	12	12	21	23	20	13
1	22	15	24	24	22	21
2	24	14	27	23	23	21
3	25	18	24	25	21	22
4	23	15	27	24	20	20
5	25	17	27	23	25	24
6	24	16	28	24	26	22
7	22	15	27	23	27	24
8	27	14	26	23	28	23
9	27	15	28	24	26	25
10	25	14	27	26	25	24
11	25	13	28	25	24	20
12	28	15	25	24	24	23
13	27	16	27	23	26	24
14	23	18	26	26	23	25
15	25	14	27	25	26	23
16	26	15	26	24	25	26
Ketoconazole	27	24	28	25	26	28

*24–14 mm = significant activity; 7–13 mm = moderate activity; < 7 mm = weak activity.[30,31]

the newly synthesized compounds ($\mathbf{L}^1$–$\mathbf{L}^4$) and their metal complexes (**1**–**16**) containing Co (II), Cu (II), Ni (II) and Zn (II) possess good biological activity. New derivatives ($\mathbf{L}^1$–$\mathbf{L}^4$) and their complexes obtained were screened for their antibacterial activity against *E. coli*, *B. subtillis*, *S. flexneri*, *S. aureus*, *P. aeruginosa* and *S. typhi* and for antifungal activity against *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani* and *C. glaberata*. The activity data exhibited a markedly 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 of $\mathbf{L}^1$–$\mathbf{L}^4$ may be rationalized on the basis of their structures, mainly possessing an additional C=N bond with heteroaromatic/aromatic system. It has been suggested that 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[44,45] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups[46–48] within the chelate ring system so formed during coordination. 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 micro-organism[49–52] thus destroying them more aggressively.

References

[1] Varma, R.S. and Khan, I.A. (1978) *Ind. J. Med. Res.* **67**, 315.
[2] Popp, F.D. and Pajouhesh, H.J. (1988) *Pharm. Sci.* **17**, 1052.
[3] Varma, R.S. and Nobles, W.L. (1975) *J. Pharm. Sci.* **64**, 881.
[4] Popp, F.D., Parson, R. and Donigan, B.E. (1980) *J. Heterocycl. Chem.* **17**, 1329.
[5] Kontz, F. (1973) *Sci. Pharm.* **41**, 123.
[6] Silver, F.P., Popp, F.D., Casey, A.C., Chakraborty, D.P., Cullen, E., Kirsch, W.R., McClesky, J.E. and Sinha, B. (1967) *J. Med. Chem.* **10**, 986.
[7] Padhya, A.C., Meth, J.J., Dighe, V.S. and Somoshkhara, S. (1973) *Sci. Cult.* **39**, 55.
[8] Popp, F.D., Parson, R. and Donigan, B.E. (1980) *J. Pharm. Sci.* **69**, 1237.
[9] Popp, F.D. (1984) *J. Heterocycl. Chem.* **21**(2), 289–291.
[10] Shukla, S.K. and Singh, S.P. (1983) *Indian J. Chem.* **22B**(7), 697–699.
[11] Scovill, J.P., Klayman, D.L. and Franchino, C.E. (1982) *J. Med. Chem.* **25**, 1261.
[12] Schmidhammer, H., Hrovat, G., Reiss, M. and Ungerank, C. (1996) *Sci. Pharm.* **64**, 655.
[13] Singh, D. and Singh, R.V. (1993) *J. Inorg. Biochem.* **15**, 227.
[14] Protivinsky, R. (1971) *Antibiot. Chemother.* **17**, 101.
[15] Joshi, K.C., Pathak, V.N. and Jain, S.K. (1980) *Pharmazie* **35**(11), 677–679.
[16] Shepherd, R.G. (1970) In: Burger, A., ed., *Medicinal Chemistry* (Wiley, New York).
[17] Heinisch, I. and Tonew, M. (1976) *Pharmazie* **31**(12), 840–845.
[18] Sing, S.P., Shukla, S.K. and Awasthi, L.P. (1983) *Curr. Sci.* **52**, 766.
[19] Kupinic, M., Medic-Saric, M., Movrin, M. and Maysinger, D. (1979) *J. Pharm. Sci.* **68**, 459.
[20] Danda, A., Kaur, V. and Singh, P. (1993) *Indian J. Pharm. Sci.* **55**, 129.
[21] Logan, J.C., Fox, M.P., Morgan, J.H., Makohon, A.M. and Pfau, C.J. (1975) *J. Gen. Virol.* **28**, 271.
[22] Omar, A., Mohsen, M.E., Nabil, H. and Hassan, M. (1984) *Arch. Pharm.* **317**(8), 668–674.

[23] Mitscher, L.A., Wai-Cheong, W., De Meulenaere, T., Sulko, J. and Darke, S. (1981) **15**, 1071.
[24] Varma, R.S., Pandey, K.R. and Kumar, P. (1982) *Ind. J. Pharm. Sci.* **44**(6), 132.
[25] Heilmeyer, L. (1967) *French Patent 5536; Chem. Abstr.* (1969) **71**, 423015.
[26] Popp, F.D., Parson, R. and Donigan, B.E. (1980) *J. Pharm. Sci.* **69**, 1235.
[27] Rajopadhye, M. and Popp, F.D. (1988) *J. Med. Chem.* **31**, 1001.
[28] El-Gendy, A.A., Nadia, A.A., El-Taher, Z.S. and Hosney, A.E. (1993) *Alexandria J. Pharm. Sci.* **7**, 99.
[29] Vogel, A.I. (1978) *A Textbook of Quantitative Inorganic Analysis*, 4th Ed. (ELBS and Longman, London).
[30] Atta-ur-Rahman, Choudhary, M.I. and Thomsen, W.J. (2001) *Bioassay Techniques for Drug Development* (Harwood Academic Publishers, The Netherlands), p 16.
[31] Khan, K.M., Saify, Z.S., Zeeshan, A.K., Ahmed, M., Saeed, M., Schick, M., Kohlbau, H.J. and Voelter, W. (2000) *Arzneim-Forsch./Drug Res.* **50**, 915.
[32] Geary, W.J. (1971) *Coord. Chem. Rev.* **7**, 81.
[33] Nakamoto, K. (1970) *Infrared Spectra of Inorganic and Coordination Compounds*, 2nd Ed. (Wiley Interscience, New York).
[34] Agarwal, R.K. (1988) *J. Indian Chem. Soc.* **65**, 448.
[35] Bellamy, L.J. (1971) *The Infrared Spectra of Complex Molecules* (John Wiley, New York).
[36] Ferrero, J.R. (1971) *Low-frequency Vibrations of Inorganic and Coordination Compounds* (John Wiley, New York).
[37] Simmons, W.W. (1978) *The Sadtler Handbook of Proton NMR Spectra* (Sadtler Research Laboratories, Inc.).
[38] Pasto, D.J. (1969) *Organic Structure Determination* (Prentice Hall International, London).
[39] Lever, A.B.P. and Lewis, J. (1963) *J. Chem. Soc.*, 2552.
[40] Carlin, R.L. (1965) *Transition Metal Chemistry*, 2nd Ed. (Marcel Decker, New York).
[41] Estes, W.E., Govel, D.P., Halfield, W.B. and Hodgson, D.J. (1978) *Inorg. Chem.* **17**, 1415.
[42] Balhausen, C.J. (1962) *An Introduction to Ligand Field* (McGraw Hill, New York).
[43] Lever, A.B.P. (1984) *Inorganic Electronic Spectroscopy* (Elsevier, Amsterdam).
[44] Chohan, Z.H., Scozzafava, A. and Supuran, C.T. (2003) *J. Enz. Inhib. Med. Chem.* **17**, 261.
[45] Chohan, Z.H. (2002) *Appl. Organomet. Chem.* **16**, 17.
[46] Chohan, Z.H., Farooq, M.A., Scozzafava, A. and Supuran, C.T. (2002) *J. Enzyme Inhib. Med. Chem.* **17**, 1.
[47] Chohan, Z.H., Rauf, A. and Supuran, C.T. (2002) *Metal-Based Drugs* **8**, 287.
[48] Chohan, Z.H., Iqbal, M.S., Iqbal, H.S., Scozzafava, A. and Supuran, C.T. (2002) *J. Enzyme Inhib. Med. Chem.* **17**, 87.
[49] Hassan, M.U., Chohan, Z.H. and Supuran, C.T. (2002) *Main Group Metal Chem.* **25**, 291.
[50] Chohan, Z.H., Pervez, H., Kausar, S. and Supuran, C.T. (2002) *Synth. React. Inorg. Met-Org. Chem.* **3**, 529.
[51] Chohan, Z.H., Pervez, H., Rauf, A. and Supuran, C.T. (2002) *Metal-Based Drugs* **8**, 42.
[52] Chohan, Z.H., Scozzafava, A. and Supuran, C.T. (2003) *J. Enzyme Inhib. Med. Chem.* **18**, 259.