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In-vitro antibacterial, antifungal and cytotoxic activities of some coumarins and their metal complexes

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Abstract

A series of new antibacterial and antifungal coumarin-derived compounds and their transition metal complexes [cobalt (II), copper (II), nickel (II) and zinc (II)] have been synthesized, characterized and 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, Candida glaberata.* The results of these studies show the metal complexes to be more antibacterial and antifungal as compared to the uncomplexed coumarins. The brine shrimp bioassay was also carried out to study their *in vitro* cytotoxic properties.

Keywords: *Coumarins, metal complexes, antibacterial, antifungal, cytotoxicity*

Introduction

Coumarins are members of the benzopyrone class, which display not only important structural variety but also significant biological [1–5] and pharmacological [6,7] properties. Many of these compounds possess antibacterial [6], antifungal [7] and insecticidal [4] activities. The diverse biological activity of various coumarins as anticoagulants [8], antithrombotics [9], HIV inhibitors [10,11] and human progesterone receptor agonists [12,13] has also been investigated. Several reports on new synthetic routes for these derivatives have been published during the last decade [14–20].

Such a variety of interesting biological activity for this class of derivatives prompted us to explore and synthesize some new heteroaromatic- and hydrazide-derived coumarins and investigate their antibacterial and antifungal activity. The ligands, along with their metal complexes were screened for 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 glaberata* respectively.

Material and methods

Solvents used were of analytical grade; all metal (II) were used as chloride salts. IR spectra were recorded on a 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 carried out the C, H and N analyses. Conductance of the metal complexes was determined in DMF on a Hitachi (Japan) YSI-32 model conduct meter. Magnetic

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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 content by EDTA titration [21]. Antibacterial, antifungal and cytotoxic screening was done at the HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Preparation of ligands (L^1–L^6) and metal (II) complexes (1–24)

3-Formyl-4-chlorocoumarin. (1) [22] Phosphorus oxychloride (10 mL) was added dropwise to a solution of dimethylformamide (DMF) (20 mL) keeping the temperature below 5°C. solution of 4-hydroxycoumarin (4.0 g) in DMF (10 mL) was then gradually added to the mixture with constant stirring and maintenance of the temperature of the reaction mixture below 5°C. The reaction mixture was then allowed to stand at room temperature for 2 h and then heated on a steam bath for 1 h. After cooling, the reaction mixture was poured onto crushed ice and neutralized with sodium carbonate. A solid product was immediately formed which was crystallized from ethanol to give a yellow solid (80%), m.p. 115°C.

Preparation of ligand (L^1)

To a stirred warm ethanolic solution (30 mL) of 4-aminopyrimidine (0.95 g, 0.01 mol) was added 3-formyl-4-chlorocoumarin (**1**) (1.1 g, 0.01 mol) in ethanol (50 mL). Then 2–3 drops of concentrated H_2SO_4 were added and the mixture refluxed for 3 h. The completion of reaction was monitored by TLC. After completion the reaction 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 (**L^1**). The same method was applied for the preparation of (**L^2–L^6**) by using the corresponding heteroaromatic amines/hydrazides, and working under the same conditions and with the same respective molar ratio.

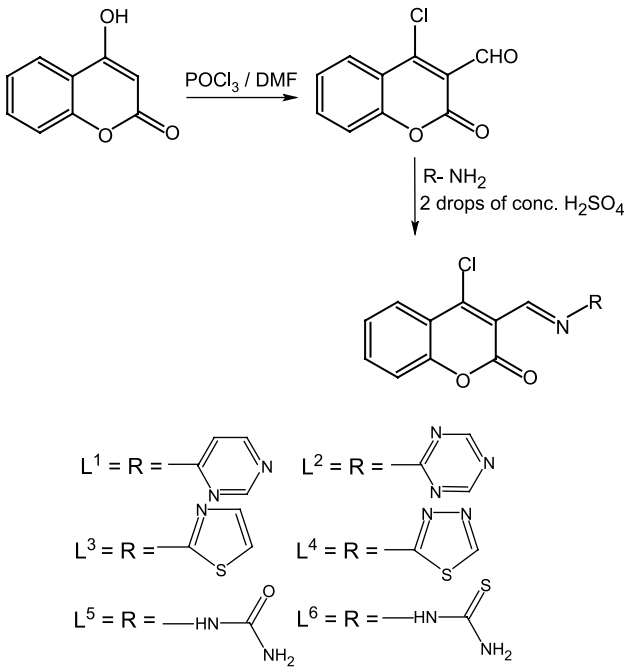
Preparation of metal (II) complexes (1–24)

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 the 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. The obtained solid 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 complexes (**1–24**) by using the respective metal (II) salts.

Biological activity

*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 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 [22]. 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 agar media using a sterile metallic borer with centers at least 24 mm. Recommended concentration (100 µl) of the test sample (1 mg/ml in DMSO) was introduced into the respective wells. Other wells were supplemented with DMSO and the 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 the zones showing complete inhibition (mm). Growth inhibition was compared with the standard drug. In order to clarify any effect of DMSO or DMF on the biological screening, separate studies were carried out with solutions alone of DMSO and DMF and they showed no activity against any bacterial strains.

*Antifungal activity (*in-vitro*)*. Antifungal activities of all compounds were studied against six fungal cultures. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspension and transferred to petri plates. Discs soaked in 20 ml (10 µg/ml in DMSO) of all compounds were placed at



Scheme 1. Synthesis of ligand (**L^1–L^6**).

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Table I. Physical, spectral and analytical data of the ligands ($\mathbf{L^1}$–$\mathbf{L^6}$).

Ligand/ Mol. Form	M.P (°C)	IR (cm^{-1})	C, H, N % Calc. (found)	Yield (%)
$\mathbf{L^1}$ [265.45] ($C_{14}H_8N_3ClO_2$)	126	1715 (C=O), 1645 (C=N)	58.9 2.8 14.7 (58.6)(3.1)(14.5)	65
$\mathbf{L^2}$ [286.45] ($C_{13}H_7N_4ClO_2$)	118	1715 (C=O), 1655 (C=N)	54.5 2.4 19.5 54.6)(2.2)(19.9)	60
$\mathbf{L^3}$ [290.55] ($C_{13}H_7N_2ClO_2S$)	130	1715 (C=O), 1650 (C=N)	53.7 2.4 9.6 (53.4)(2.6)(9.5)	63
$\mathbf{L^4}$ [291.55] ($C_{12}H_6N_3ClO_2S$)	122	1715 (C=O), 1650 (C=N)	49.4 2.1 14.4 (49.8)(2.3)(14.0)	62
$\mathbf{L^5}$ [265.45] ($C_{11}H_8N_3ClO_3$)	135	1735, 1715 (C=O), 1650 C=N) 1425 (N—N)	49.7 3.0 15.8 (49.5)(3.4)(15.6)	65
$\mathbf{L^6}$ [281.55] ($C_{11}H_8N_3ClO_2S$)	128	1715 (C=O), 1655 (C=N), 1415 (N—N), 1350, 1155 (S=O)	46.9 2.8 14.9 (47.3)(2.9)(14.7)	63

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 the standard drugs miconazole and amphotericin B.

Minimum inhibitory concentration (MIC). Compounds showing promising antibacterial/antifungal activity were selected for minimum inhibitory concentration studies. The minimum inhibitory concentration was determined using the disc diffusion technique by preparing discs containing 10, 25, 50 and 100 μg/ml of the compounds and applying the reported protocol [23].

Table II. ^{1}H NMR data of the ligands ($\mathbf{L^1}$–$\mathbf{L^6}$) and their Zn (II) complexes (**19**–**24**).

No	^{1}H NMR (DMSO-d_6)(ppm)
$\mathbf{L^1}$	6.7 (1H, s, CH=N), 7.3–7.7 (4H, m, ar-H), 7.8–8.1(3H, m, pyrm-H)
$\mathbf{L^2}$	6.8 (1H, s, CH=N), 7.3–7.7 (4H, m, ar-H), 8.1–8.2(2H, m, triaz-H)
$\mathbf{L^3}$	6.7 (1H, s, CH=N), 7.3–7.7(4H, m, ar-H), 8.0–8.2(2H, m, thiaz-H)
$\mathbf{L^4}$	6.8 (1H, s, CH=N), 7.3–7.7 (4H, m, ar-H), 8.3(1H, s, thiadiaz-H)
$\mathbf{L^5}$	6.7 (1H, s, CH=N), 7.3–7.5 (4H, m, ar-H), 9.8 (2H, s, NH_2), 11.8 (1H, s, NH)
$\mathbf{L^6}$	6.7 (1H, s, CH=N), 7.3–7.5 (4H, m, ar-H), 9.7 (2H, s, NH_2), 11.8 (1H, s, NH)
19	6.8 (1H, s, CH=N), 7.7–8.0 (4H, m, ar-H), 8.2–8.5(3H, m, pyrm-H)
20	6.9 (1H, s, CH=N), 7.6–7.9 (4H, m, ar-H), 8.5–8.7(2H, m, triaz-H)
21	6.9 (1H, s, CH=N), 7.5–7.8(4H, m, ar-H), 8.3–8.5(2H, m, thiaz-H)
22	6.8 (1H, s, CH=N), 7.5–7.9 (4H, m, ar-H), 8.5 (1H, s, thiadiaz-H)
23	6.9 (1H, s, CH=N), 7.5–7.8 (4H, m, ar-H), 10.2 (2H, s, NH_2), 11.9 (1H, s, NH)
24	6.9 (1H, s, CH=N), 7.3–7.5 (4H, m, ar-H), 10.1 (2H, s, NH_2), 11.9 (1H, s, NH)

*Cytotoxicity (*in-vitro*)*. Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm) filled with artificial seawater, which was prepared with 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 minor compartment was open to ordinary light. After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 ml of DMF. From this stock solutions 500, 50 and 5 μg/ml were transferred to 9 vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of 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 Finney computer program to determine the LD_{50} values [24].

Results and discussion

Chemistry

The methodology adopted was to first convert 4-hydroxycoumarin into its 4-chloro-3-formyl derivative in the presence of phosphoryl chloride and dimethylformamide (DMF). The aldehyde was then condensed with heterocyclic amines, semicarbazide or thiosemicarbazide leading to a new series of Schiff's bases (Scheme 1). The synthesized compounds were further used to prepare their cobalt (II), copper (II), nickel (II) and zinc (II) metal complexes (**1**–**24**) which were all characterized by IR, NMR, UV–Visible, molar conductance, magnetic moment and elemental analyses data. The ligands ($\mathbf{L^1}$–$\mathbf{L^6}$) were prepared by refluxing the appropriate amount of an ethanolic solution of 3-formyl-4-chlorocoumarin with the corresponding heteroaromatic amines/hydrazides, in a 1:1 molar

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

No	Metal complex/ Mol. Formula	M.P (°C)	B.M (μ_{eff})	C, H, N; Calc. (found) %	Yield (%)
1	$[Co(L^1)_2]Cl_2$ [665.25] ($C_{28}H_{16}CoCl_3N_6O_4$)	218–220	3.9	50.5 (50.8), 2.4 (2.9), 12.6 (12.2)	70
2	$[Co(L^2)_2]Cl_2$ [667.25] ($C_{26}H_{14}CoCl_3N_8O_4$)	222–224	4.1	46.8 (46.3), 2.1 (2.2), 16.8 (16.4)	72
3	$[Co(L^3)_2]Cl_2$ [675.45] ($C_{26}H_{14}CoCl_3N_4O_4S_2$)	195–197	4.0	46.2 (46.8), 2.1 (2.3), 8.3 (8.4)	70
4	$[Co(L^4)_2]Cl_2$ [677.45] ($C_{24}H_{12}CoCl_3N_6O_4S_2$)	220–222	4.1	42.5 (42.6), 1.8 (1.3), 12.4 (12.3)	71
5	$[Co(L^5)_2]Cl_2$ [625.25] ($C_{22}H_{16}CoCl_3N_6O_6$)	226–228	4.1	42.2 (42.6), 2.6 (2.8), 13.4 (13.3)	69
6	$[Co(L^6)_2]Cl_2$ [657.45] ($C_{22}H_{16}CoCl_3N_6O_4S_2$)	216–218	4.0	40.2 (40.6), 2.4 (2.1), 12.8 (12.3)	70
7	$[Cu(L^1)_2]Cl_2$ [669.9] ($C_{28}H_{16}CuCl_3N_6O_4$)	222–224	1.4	50.2 (50.5), 2.4 (2.1), 12.5 (12.0)	68
8	$[Cu(L^2)_2]Cl_2$ [671.9] ($C_{26}H_{14}CuCl_3N_8O_4$)	222–224	1.6	46.4 (46.6), 2.1 (2.4), 16.7 (16.5)	70
9	$[Cu(L^3)_2]Cl_2$ [680.1] ($C_{26}H_{14}CuCl_3N_4O_4S_2$)	218–220	1.4	45.9 (45.7), 2.1 (2.5), 8.2 (8.4)	69
10	$[Cu(L^4)_2]Cl_2$ [682.1] ($C_{24}H_{12}CuCl_3N_6O_4S_2$)	224–226	1.6	42.2 (42.3), 1.8 (1.5), 12.3 (12.0)	71
11	$[Cu(L^5)_2]Cl_2$ [629.9] ($C_{22}H_{16}CuCl_3N_6O_6$)	218–220	1.5	41.9 (41.6), 2.5 (2.8), 13.3 (13.5)	71
12	$[Cu(L^6)_2]Cl_2$ [662.1] ($C_{22}H_{16}CuCl_3N_6O_4S_2$)	220–224	1.6	39.9 (40.3), 2.4 (2.5), 11.7 (11.3)	71
13	$[Ni(L^1)_2]Cl_2$ [665.1] ($C_{28}H_{16}NiCl_3N_6O_4$)	220–222	3.2	50.5 (50.6), 2.4 (2.7), 12.6 (12.5)	69
14	$[Ni(L^2)_2]Cl_2$ [667.1] ($C_{26}H_{14}NiCl_3N_8O_4$)	225–227	3.3	46.8 (46.5), 2.1 (2.2), 16.8 (16.3)	68
15	$[Ni(L^3)_2]Cl_2$ [675.3] ($C_{26}H_{14}NiCl_3N_4O_4S_2$)	218–220	3.2	46.2 (46.5), 2.1 (2.2), 8.3 (8.1)	70
16	$[Ni(L^4)_2]Cl_2$ [677.3] ($C_{24}H_{12}NiCl_3N_6O_4S_2$)	223–225	3.4	42.5 (42.2), 1.8 (1.7), 12.4 (12.7)	68
17	$[Ni(L^5)_2]Cl_2$ [625.1] ($C_{22}H_{16}NiCl_3N_6O_6$)	223–225	3.3	42.2 (42.0), 2.6 (2.2), 13.4 (13.7)	68
18	$[Ni(L^6)_2]Cl_2$ [657.3] ($C_{22}H_{16}NiCl_3N_6O_4S_2$)	223–225	3.2	40.2 (40.0), 2.4 (2.7), 12.8 (12.4)	68
19	$[Zn(L^1)_2]Cl_2$ [671.8] ($C_{28}H_{16}ZnCl_3N_6O_4$)	226–228	Dia	50.0 (50.2), 2.4 (2.0), 12.5 (12.3)	67
20	$[Zn(L^2)_2]Cl_2$ [673.9] ($C_{26}H_{14}ZnCl_3N_8O_4$)	220–222	Dia	46.3 (46.5), 2.1 (2.4), 16.6 (16.4)	71
21	$[Zn(L^3)_2]Cl_2$ [682.0] ($C_{26}H_{14}ZnCl_3N_4O_4S_2$)	218–220	Dia	45.7 (45.4), 2.1 (2.8), 8.2 (8.4)	70
22	$[Zn(L^4)_2]Cl_2$ [684.0] ($C_{24}H_{12}ZnCl_3N_6O_4S_2$)	224–226	Dia	42.1 (42.4), 1.8 (2.0), 12.3 (12.4)	67
23	$[Zn(L^5)_2]Cl_2$ [631.8] ($C_{22}H_{16}ZnCl_3N_6O_6$)	222–224	Dia	41.8 (41.4), 2.5 (2.8), 13.3 (13.4)	67
24	$[Zn(L^6)_2]Cl_2$ [664.0] ($C_{22}H_{16}ZnCl_3N_6\ O_4S_2$)	225–227	Dia	39.8 (39.5), 2.4 (2.9), 12.7 (12.3)	68

ratio. The structures of these synthesized ligands was established by their IR, NMR and microanalytical data (Tables I and II). All metal complexes (**1**–**24**) (Table III) of these ligands were prepared by the stoichiometric reaction of the corresponding ligand with the respective metal (II) salt as chloride in a molar ratio M:L of 1:2. All the complexes were air and moisture stable and were are intensely colored amorphous solids which decomposed without melting. They were insoluble in common organic solvents and only soluble in water, DMF and DMSO. Molar conductance values of the soluble complexes in DMF (10^3 M solution at 25°C), indicated high values (86–95 ohm^{-1} cm^{-2} mol^{-1}) suggesting that they were all electrolytic in nature [25].

The elemental analyses data agree well with the proposed formulae for the ligands and metal (II)

Table IV. Spectral data of the metal complexes (**1**–**24**).

No	IR (cm^{-1})	λ_{max} (cm^{-1})
1	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	7,280, 17,265, 20,485, 27,175.
2	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	7,375, 17,280, 20,690, 28,360
3	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	7,315, 17,285, 20,580, 28,275.
4	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	7,285, 17,260, 20,625, 27,390.
5	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	7,370, 17,385, 20,625, 27,485.
6	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	7,295, 17,275, 20,675, 28,295.
7	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O)	14,710, 19,575, 30,245
8	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O)	15,215, 19,265, 30,275
9	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O)	14,885, 19,315, 30,220
10	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O)	14,715, 19,385, 30,270
11	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O)	15,185, 19,510, 30,285
12	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O)	14,895, 19,405, 30,190
13	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	10,275, 15,740, 26,365, 30,170
14	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	10,315, 15,720, 26,480, 30,155
15	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	10,290, 15,745, 26,475, 30,225
16	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	10,315, 15,690, 26,555, 30,235
17	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	10,280, 15,735, 26,395, 29,995
18	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	10,295,15,580, 26,370, 29,910
19	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	28,275
20	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	29,380
21	1700 (C=O), 1635 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	28,555
22	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	29,225
23	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	29,195
24	1700 (C=O), 1640 (C=N), 435 (M—N), 415 (M—O), 315(M—Cl)	28,905.00

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Table V. *In-vitro* antibacterial 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)*	*(g)*	*(h)*	*(j)*	*(k)*
$\mathbf{L^1}$	16	15	11	16	12	15	6	5	14	12
$\mathbf{L^2}$	16	18	14	17	16	18	8	10	12	13
$\mathbf{L^3}$	18	18	16	18	18	15	12	13	14	15
$\mathbf{L^4}$	18	15	18	19	19	18	15	14	15	17
$\mathbf{L^5}$	15	12	12	15	18	14	6	10	12	12
$\mathbf{L^6}$	16	14	10	15	15	14	6	10	11	15
1 $Co(L^1)_2Cl_2$	18	22	21	20	21	22	18	15	18	19
2 $Co(L^2)_2Cl_2$	20	20	20	22	22	21	16	16	17	20
3 $Co(L^3)_2Cl_2$	20	22	21	20	22	21	19	17	20	20
4 $Co(L^4)_2Cl_2$	13	20	19	20	21	22	15	15	21	19
5 $Co(L^5)_2Cl_2$	18	21	21	20	22	20	18	14	18	20
6 $Co(L^6)_2Cl_2$	20	22	21	20	22	20	15	15	20	20
7 $Cu(L^1)_2Cl_2$	21	20	22	24	23	22	16	14	18	21
8 $Cu(L^2)_2Cl_2$	21	25	20	22	23	22	18	18	19	21
9 $Cu(L^3)_2Cl_2$	20	23	20	23	22	20	16	15	20	20
10 $Cu(L^4)_2Cl_2$	20	22	20	24	21	21	18	15	18	20
11 $Cu(L^5)_2Cl_2$	18	22	22	22	21	23	15	16	18	19
12 $Cu(L^6)_2Cl_2$	20	20	20	22	21	20	14	15	18	19
13 $Ni(L^1)_2Cl_2$	21	20	22	25	21	22	15	15	18	19
14 $Ni(L^2)_2Cl_2$	20	22	22	23	23	22	18	16	19	18
15 $Ni(L^3)_2Cl_2$	18	23	22	24	22	22	15	15	18	18
16 $Ni(L^4)_2Cl_2$	22	22	20	23	22	21	15	14	18	19
17 $Ni(L^5)_2Cl_2$	20	20	22	22	22	21	18	12	18	18
18 $Ni(L^6)_2Cl_2$	18	22	20	22	22	21	19	15	18	18
19 $Zn(L^1)_2Cl_2$	18	20	22	22	21	21	18	14	20	20
20 $Zn(L^2)_2Cl_2$	20	22	21	22	23	22	16	15	17	19
21 $Zn(L^3)_2Cl_2$	21	22	19	22	21	20	15	15	18	20
22 $Zn(L^4)_2Cl_2$	22	20	19	22	22	23	15	14	17	20
23 $Zn(L^5)_2Cl_2$	20	21	20	22	21	23	16	12	18	19
24 $Zn(L^6)_2Cl_2$	20	24	20	22	21	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) = *Escherichia coli*, (b) = *Klebsiella pneumoniae*, (c) = *Proteus mirabilis*, (d) = *Pseudomonas aeruginosa*, (e) = *Salmonella typhi*, (f) = *Shigella dysenteriae*, (g) = *Bacillus cereus*, (h) = *Corynebacterium diphtheriae*, (j) = *Streptococcus pyogenes*, (k) = *Staphylococcus aureus*. Imipenum = standard drug.

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complexes. 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 III. The IR spectra of the complexes showed a lower shift of wave numbers in ν(C=N) of the azomethine by 10–20 cm^{-1}, respectively. The band located at 1715 cm^{-1} in all the ligands attributed [26] to ν (C=O) moiety of the coumarin also moved to a lower frequency by 15–20 cm^{-1} on coordination. This data on comparison with the spectra of the ligands suggests that the azomethine-N and coumarin-O of the ligands are only involved in coordination with the metal ions. Also, a new band at 315 cm^{-1}, suggesting [27] coordination of ν(M—Cl) in the Co (II), Ni (II) and Zn (II) complexes was observed while in the spectra of the Cu (II) complexes this band was not observed indicating an octahedral geometry for the Co (II), Ni (II) and Zn (II) complexes and a square-planar geometry for the Cu (II) complexes. The far IR spectra of these metal complexes (Table II) exhibited new bands, which are not present in the spectra of the ligands. These bands are located at 435 and 415 cm^{-1}, and are assigned [28,29] to ν(M—N) of azomethine-N and ν(M=O) of coumarin-O, so supporting evidence for the bonding of the ligands with the metal ions. Accordingly, the above data suggests that the ligands behave as bidentate towards all metals.

NMR spectra. The ^{1}H NMR spectra of the free ligands and their diamagnetic Zn (II) chelates were determined in DMSO-d_6. The ^{1}H NMR spectral data are reported along with the possible assignments in Table II. All the protons were found as to be in their expected region [29]. The conclusions drawn from these studies lend further support to the mode of bonding discussed in their IR spectra. In the spectra of the diamagnetic Zn (II) complexes, these protons shifted downfield due to the increased conjugation and coordination to the metal atoms [30]. The number of protons calculated from the integration

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Table VI. *In-vitro* antifungal activity data for 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)*
$\mathbf{L}^1$	16	17	11	16	12	11
$\mathbf{L}^2$	15	12	14	14	15	14
$\mathbf{L}^3$	16	15	12	15	10	15
$\mathbf{L}^4$	15	15	11	15	10	15
$\mathbf{L}^5$	16	14	11	14	12	14
$\mathbf{L}^6$	16	15	11	15	12	12
1	18	15	15	18	22	18
2	18	12	15	18	20	20
3	20	24	15	20	18	18
4	18	12	14	20	20	20
5	21	11	15	18	6	21
6	20	18	14	20	20	18
7	20	18	16	21	18	20
8	18	16	16	18	18	20
9	20	15	18	20	15	18
10	18	16	15	18	20	15
11	20	15	15	20	18	20
12	18	14	18	20	18	20
13	20	16	14	21	20	18
14	21	17	18	18	18	18
15	20	18	16	20	18	18
16	18	18	16	18	20	16
17	18	18	18	18	21	18
18	20	14	15	20	22	20
19	20	13	16	20	18	18
20	21	15	16	20	20	16
21	20	11	20	15	21	18
22	18	16	18	20	22	20
23	20	14	20	18	18	18
24	18	11	16	20	18	18
Miconazole	30	20	25	25	30	25
Amphotericin B	30	25	30	25	30	30

Ligand: > 14 mm = significant activity; 7–13 mm = moderate activity; < 7 mm = weak activity. (a) = *Trichophyton longifusus*, (b) = *Candida albicans*, (c) = *Aspergillus flavus*, (d) = *Microsporum canis*, (e) = *Fusarium solani*, (f) = *Candida glaberata*. Miconazole and Amphotericin B = standard drugs.

curves, and those obtained from the values of the expected CHN analyses agreed.

Electronic spectra. The Co(II) complexes exhibited well-resolved, low-energy bands at 7,280–7,375 cm^{-1}, 17,260–17,385 cm^{-1} and a strong high-energy band at 20,485–20,675 cm^{-1} (Table IV) which are assigned [32] to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ for a high-spin octahedral geometry [32]. A high intensity band at 27,175–28,360 cm^{-1} was assigned to the metal → ligand charge transfer. The magnetic susceptibility measurements (3.9–4.1 B.M) for the solid Co (II) complexes are also indicative of three unpaired electrons per Co (II) ion suggesting [33–41] consistency with their octahedral environment. The electronic spectra of the Cu (II) complexes (Table IV) showed two low-energy weak bands at 14,710–15,215 cm^{-1} and 19,265–19,575 cm^{-1} and a strong high-energy band at 30,190–30,285 cm^{-1} which was 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 → ligand charge transfer. Also, the magnetic moment values (1.4–1.6 B.M) (Table II) for the copper (II) are indicative of anti-ferromagnetic spin-spin interaction through molecular association [43,44]. The electronic spectra of the Ni (II) complexes showed d-d bands in the region 10,275–10,315, 15,580–15,740 and 26,365–26,555 cm^{-1}. These are assigned [42] 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 29,910–30,235 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements (3.2–3.4 B.M) showed two unpaired electrons per Ni (II) ion suggesting [44] also an octahedral geometry for the Ni (II) complexes. The electronic spectra of the Zn (II) complexes exhibited only a high-intensity band at 28,275–29,380 cm^{-1} and are assigned [42] to a ligand → metal charge transfer.

Biological activity

Antibacterial bioassay

All compounds were tested against *E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, S. typhi, S. dysenteriae, B. cereus, C. diphtheriae, S. aureous* and *S. pyogenes* bacterial strains (Table V) according to literature protocol [22,23]. 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

The antifungal screening of all compounds was carried out against *T. longifusus, C. albican, A. flavus, M. canis, F. solani* and *C. glaberate* fungal strains according to the literature protocol [23]. The results were compared with the standard drugs miconazole and amphotericin B. The results given in Table VI indicate that all ligands were active against one or more fungal species however, the metal (II) complexes (**1**–**24**) of these compounds 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. [38] It was observed that

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Table VII. Minimum inhibitory concentration (μg/ml) of selected compounds against selected bacteria.

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

(a) = *Escherichia coli*, (b) = *Klebsiella pneumoniae*, (c) = *Proteus mirabilis*, (d) = *Pseudomonas aeruginosa*, (e) = *Salmonella typhi*, (f) = *Shigella dysenteriae*, (g) = *Bacillus cereus*, (h) = *Corynebacterium diphtheriae*, (j) = *Streptococcus pyogenes*, (k) = *Staphylococcus aureus*.

only ligands $\mathbf{L}^5$ and $\mathbf{L}^6$ and the Cu (II) and Ni (II) metal complexes (**11**, **12**, **17** & **18**) displayed a weak cytotoxic activity against *Artemia salina*, while the other compounds gave values of LD_{50} (1000 in this assay, and therefore can be considered to be non-cytotoxic.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of some selected compounds, which showed significant activity against selected bacterial species, was determined using the disc diffusion method [23]. The MIC of these compounds varies from 10–100 μg/ml. The results shown in Table VII indicated that these compounds were the most active in inhibiting the growth of the tested organisms at a 10 μg/ml concentration.

The biological activity of the ligands exhibited a markedly enhancement on coordination with the metal ions against all the test bacterial/fungal strains. The ligands 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 (**$\mathbf{L}^1$–$\mathbf{L}^6$**) may be rationalized on the basis that their structures mainly possess an additional C=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 metal ions on coordination. Moreover, coordination reduces the polarity [36,37] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups [38–43] within the chelate ring system formed during coordination. This process, in turn, increases the lipophilic nature of the central metal atom, which favors its permeation more efficiently through the lipid layer of the micro-organism [44–54] thus destroying them more aggressively.

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