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Binding of Transition Metal Ions [Cobalt, Copper, Nickel and Zinc] with Furanyl-, Thiophenyl-, Pyrrolyl-, Salicylyl- and Pyridyl-Derived Cephalexins as Potent Antibacterial Agents

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A method is described for the preparation of novel cephalexin-derived furanyl-, thiophenyl-, pyrrolyl-, salicylyl- and pyridyl-containing compounds showing potent antibacterial activity. The binding of these newly synthesized antibacterial agents with metal ions such as cobalt(II), copper(II), nickel(II) and zinc(II) has been studied and their inhibitory properties against various bacterial species such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* are also reported. These results suggest that metal ions to possess an important role in the designing of metal-based antibacterials and that such complexes are more effective against infectious diseases compared to the uncomplexed drugs.

Keywords: antibacterials; metal complexes; cephalexin

INTRODUCTION

Most antibiotics used clinically act as inhibitors of bacterial cell wall biosynthesis or affect protein synthesis on ribosomes without intervening in the metabolic processes of the pathogens.[1] The bacteria have developed strategies for neutralizing plasma-derived protease inhibitors and use proteases for other critical processes[2] such as colonization, evasion of host immune defenses, acquisition of nutrients for growth and tissue damage during infection. These are all consequences of the indiscriminate use of antibiotics, with the consequent emergence of resistance to the clinically used agents.[1,2] As a result, drug resistance to the presently available classes of antibiotics is globally becoming a relevant medical problem. A possible solution to this problem might be the process of chelation *via* coordination with metal ions, which may constitute one emerging prospect for the design and development of novel metal-based antibiotics.[3–5] In an effort to explore the role of metal-based drugs as potential antibacterials, we have commenced[6–14] an extensive program to synthesize novel antibacterial compounds and to study their therapeutic potential upon chelation/coordination with metal ions. We chose cephalexin, a clinically well-known and important antibiotic for the present study. The free amino group of cephalexin was initially condensed with furan-2-, thiophene-2-, pyrrole-2-, pyridine-2-aldehydes and salicylaldehyde to afford Schiff's bases ($\mathbf{L^1}$–$\mathbf{L^5}$, Scheme 1) and then complexed with four transition metal ions *i.e.* cobalt(II), copper(II) nickel(II) and zinc(II). The structures of the newly synthesized ligands ($\mathbf{L^1}$–$\mathbf{L^5}$) and their corresponding complexes with all four metal ions were determined using different spectroscopic and microanalytical techniques. Molecular models of the newly synthesized compounds ($\mathbf{L^1}$–$\mathbf{L^5}$) indicate that their structures contain potential donor sites suitable for chelation with these metal ions. In order to evaluate the biological potential of the cephalexin derivatives the condensed and chelated compounds were

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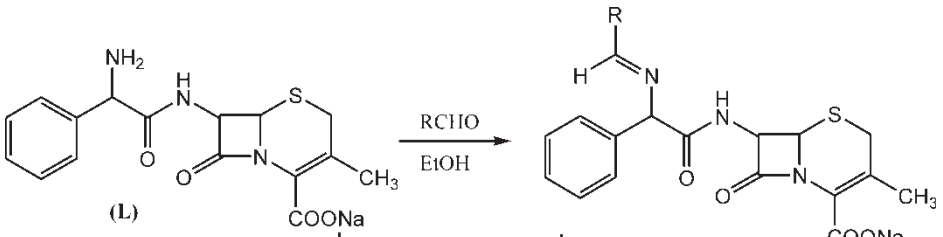
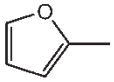
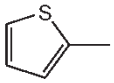
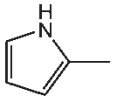
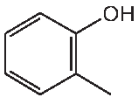
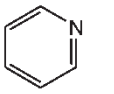


Compound	R	Yields %
L^1		67
L^2		65
L^3		62
L^4		68
L^5		65

SCHEME 1 Cephalexin-derived ligands (L^1–L^5).

subjected to antibacterial screening against four bacterial strains, of which three were Gram negative *i.e. Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* and one Gram positive *i.e. Staphylococcus aureus*.

MATERIAL AND METHODS

Cephalexin sodium salt was obtained from Pharmagen Beximco Ltd, Pakistan. Solvents used were analytical grades; all metal (II) were used as their chloride salts. IR spectra were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer. 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 conductometer. Magnetic measurements were carried out on solid complexes using the Gouy's method. Melting points were recorded on a Gallenkamp (U.K.) apparatus and are not corrected.

Preparation of Cephalexin-Derived Schiff Bases (L^1–L^5) and Metal(II) Complexes

To a stirred solution of cephalexin sodium salt, **L**, (4.5 g, 0.01 mmol) in warm ethanol (20 mL) was added furan-2-carboxaldehyde (0.97 g, 0.01 mmol) in ethanol (10 mL). The mixture was refluxed for 2 h and completion of reaction was monitored through TLC. After completion of reaction the mixture was cooled to afford a solid product. The solid residue was filtered, washed with ethanol, then with ether and dried. Crystallization from hot ethanol gave $\mathbf{L^1}$.

The same method was applied for the preparation of $\mathbf{L^2}$–$\mathbf{L^5}$ (Scheme 1) by using the corresponding aldehyde reagents, working under the same conditions and molar ratio. A solution (30 mL) of the corresponding ligand (0.02 mmol) in hot ethanol was added to a stirred solution of metal (II) chloride (0.01 mmol) in ethanol (25 mL). The mixture was refluxed for 1 h 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 complexes. The same method was used for the preparation of all other complexes by using the respective metal(II) salts.

Biological Activity

All the synthesized ligands and their corresponding metal(II) chelates were screened *in vitro* for their antibacterial activity against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Klebsiella pneumoniae* (ATCC 5836) using the agar well diffusion method[15–17]. Two to eight hours old bacterial

TABLE I Physical, Spectral and Analytical Data for the Ligands $\mathbf{L^1}$–$\mathbf{L^5}$

Ligand	M.P (°C)	IR (cm^{-1})	C, H, N; Cal. (Found) %	Yield (%)
L^1 ($C_{21}H_{18}N_3O_5NaS$)	197	3520, 1760, 1725, 1660, 1640, 1620, 1600, 1565, 1110, 945	56.4(56.7), 3.6(3.3), 9.4(10.2)	67
L^2 ($C_{21}H_{18}N_3O_4NaS_2$)	188	3515, 1760, 1725, 1660,1635, 1620, 1600, 1565, 1115, 950	54.4 (54.6), 3.9 (3.2), 9.1 (8.8)	65
L^3 ($C_{21}H_{19}N_4O_4NaS$)	190	3525, 1760, 1725, 1660,1640, 1620, 1600, 1565, 1115, 950	56.5 (56.9), 4.3 (4.0), 12.6 (12.8)	62
L^4 ($C_{23}H_{20}N_3O_5NaS$)	195	3575, 3520, 1760, 1725,1660, 1640, 1620, 1600, 1565, 1115	58.4 (58.8), 4.2 (3.9), 8.9 (9.3)	68
L^5 ($C_{22}H_{19}N_4O_4NaS$)	193	3518, 1760, 1725, 1660, 1640, 1620, 1560, 1115, 940	57.6 (57.4), 4.1 (4.5), 12.2 (12.6)	65

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 centres at least 24 mm. A concentration (100 μl) of the test sample (1 mg/ml in DMSO) was introduced in 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 standard drug.

RESULTS AND DISCUSSION

Chemistry

The ligands ($\mathbf{L^1}$–$\mathbf{L^5}$) were prepared by refluxing the appropriate amount of cephalexin sodium salt with the corresponding furan-, thiophene-, pyrrole, pyridine-2-carboxaldehyde and salicylaldehyde in ethanol, in a 1:1 molar ratio. The structures of the synthesized compounds/ligands were established with by IR and microanalytical data. All metal complexes (**1**–**20**) of these ligands were air and moisture stable and prepared by the stoichiometric reaction of the corresponding metal(II) chloride with the ligand, in a molar ratio M:L of 1:2. These complexes are intensely colored and 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 show low values ($16–20\ ohm^{-1}cm^{-2}mol^{-1}$) indicating that they are all non-electrolytic in nature.[18] The elemental analyses data agreed well with the proposed formulae for the ligands and also confirmed the ML_2 composition of the metal (II) chelates. Efforts to grow good crystals of the ligands and their metal chelates for X-ray diffraction studies were unsuccessful due to their poor solubility in common organic solvents.

IR Spectra

IR spectra of the ligands reported in Table I show the absence of bands at 3420 and 1715 cm^{-1} due to amino ν (NH_2) and ν (HC═O) stretching vibrations and instead, the appearance of a strong new band at ~1620 cm^{-1} assigned as azomethine, ν (HC═N) vibration indicated that the starting materials had been converted into the corresponding[19] Schiff's base ligands. The comparison of the IR spectra of the ligands with their metal complexes (Table II) indicated that all the ligands were tridentately coordinated to the cobalt, nickel and zinc and, bidentately to the copper metal ions. In addition, there appeared[20] an intense band at ~1760 cm^{-1} attributed to ν C═O stretching vibration of the β-lactam ring. This band is not shifted with respect to the ligand indicating that the β-lactam ring is not modified in the complexes, and this group does not interact directly with the metal atom. The band assigned to the amide at ~1665 cm^{-1} stretching vibration in the ligand is not shifted in the complex indicating that the amido group is not involved in coordination with the metal ion. The band at ~1600 cm^{-1} includes the stretching vibration of ν C═O of the carboxylate group in the ligand. This band has shifted to the lower frequency side by 5 cm^{-1} indicating direct participation of the carboxylate oxygen in the bonding linkage with the metallic ion. The band at ~1620 cm^{-1} due to the azomethine linkage was also shifted to lower frequency ~1610 cm^{-1} indicating[21] coordination of the azomethine nitrogen to the metal atom. However, further conclusive evidence of the coordination of the ligands with the metal atoms was established[22] by far IR spectra where the appearance of new bands at 340–355, 415–430 and 545–560 cm^{-1} assigned to ν M-S, ν M-O and ν M-N in the spectra of the metal complexes were observed; these are not observable in the spectra of their corresponding ligands. These shifts were, however, not observed in the copper (II) chelates that clearly revealed no participation of newly incorporated ligands heteroatoms to the metal ions. Also, a band at ~3525 cm^{-1} attributed to ν OH in the ligand L^4 disappeared in its metal complexes and instead appearance of a band at 1580 cm^{-1} indicated deprotonation of the OH moiety during coordination.

Electronic Spectra

The Co (II) complexes exhibited well-resolved, low-energy bands at 7,570–7,715 cm^{-1} and 17,350–17,485 cm^{-1} and a strong high-energy band at

TABLE II Physical and Analytical Data of the Metal (II) Complexes **1**–**20**

	Metal chelate, M_r, Mol. formula	M.P (°C)	B.M (μ_{eff})	C, H, N; Calc. (Found) %	Yield (%)
1	$[Co(L^1)_2]$, [907.1], ($C_{42}H_{36}N_6CoO_{10}S_2$)	221–223	3.9	55.6 (55.9), 4.0 (3.7), 9.3 (9.7)	56
2	$[Co(L^2)_2]$, [939.2], ($C_{42}H_{36}N_6CoO_8S_4$)	227–229	3.8	53.7 (53.9), 3.8 (4.2), 8.9(8.4)	60
3	$[Co(L^3)_2]$, [905.1], ($C_{42}H_{38}CoN_8O_8S_2$)	220–222	4.1	55.7 (56.1), 4.2 (4.1), 12.4 (12.2)	58
4	$[Co(L^4)_2]$, [959.1], ($C_{46}H_{40}CoN_6O_{10}S_2$)	214–216	4.2	57.5 (57.8), 4.2 (4.0), 8.8 (9.2)	55
5	$[Co(L^5)_2]$, [929.1], ($C_{44}H_{38}CoN_8O_8S_2$)	211–213	3.8	56.8 (56.5), 4.1(4.4), 12.1 (11.8)	59
6	$[Cu(L^1)_2]$, [911.1], ($C_{42}H_{36}N_6CuO_{10}S_2$)	225–227	1.8	55.3 (55.7), 4.0 (3.8), 9.2 (9.3)	61
7	$[Cu(L^2)_2]$, [943.2], ($C_{42}H_{36}N_6CuO_8S_4$)	226–228	1.6	53.4(53.5), 3.8 (3.3), 8.9 (8.6)	58
8	$[Cu(L^3)_2]$, [909.1], ($C_{42}H_{38}CuN_8O_8S_2$)	222–223	1.4	55 (55.7), 4.2 (4.0), 12.3 (12.5)	57
9	$[Cu(L^4)_2]$, [963.1], ($C_{246}H_{40}CuN_6O_{10}S_2$)	228–230	1.7	57.3 (57 1), 4.2 (4.5), 8.7 (8.9)	59
10	$[Cu(L^5)_2]$, [933.1], ($C_{44}H_{38}CuN_8O_8S_2$)	217–219	1.6	56.6 (56.8), 4.1 (3.8), 12.0 (12.4)	60
11	$[Ni(L^1)_2]$, [906.1], ($C_{42}H_{36}N_6NiO_{10}S_2$)	220–222	3.1	55.6 (55.9), 4.0 (4.2), 9.3 (9.1)	62
12	$[Ni(L^2)_2]$, [938.2], ($C_{42}H_{36}N_6NiO_8S_4$)	215–218	3.3	53.7 (53 4), 3.8 (4.0), 10.0 (10.4)	58
13	$[Ni(L^3)_2]$, [904.1], ($C_{42}H_{38}NiN_8O_8S_2$)	223–225	3.4	55.7 (55.5), 4.2 (4.6), 12.4 (12.6)	57
14	$[Ni(L^4)_2]$, [958.1], ($C_{246}H_{40}NiN_6O_{10}S_2$)	218–220	3.2	57.6 (57.8), 4.2 (4.4), 8.8 (8.5)	58
15	$[Ni(L^5)_2]$, [928.1], ($C_{44}H_{38}NiN_8O_8S_2$)	220–222	3.3	56.9 (57 3), 4.1 (4.3), 12.1 (12.5)	60
16	$[Zn(L^1)_2]$, [913.1], ($C_{42}H_{36}N_6ZnO_{10}S_2$)	217–218	Dia	55.2 (55.5), 3.9 (4.2), 9.2 (9.5)	56
17	$[Zn(L^2)_2]$, [945.2], ($C_{42}H_{36}N_6ZnO_8S_4$)	222–224	Dia	53.3 (53.5), 3.8 (4.1), 8.9 (9.2)	58
18	$[Zn(L^3)_2]$, [911.1], ($C_{42}H_{38}ZnN_8O_8S_2$)	218–220	Dia	55.3 (55.5), 4.2 (4.5), 12.3 (12.0)	60
19	$[Zn(L^4)_2]$, [965.1], ($C_{246}H_{40}ZnN_6O_{10}S_2$)	215–217	Dia	57.2 (57.0), 4.1 (4.3), 8.7 (8.8)	57
20	$[Zn(L^5)_2]$, [935.1], ($C_{44}H_{38}ZnN_8O_8S_2$)	212–214	Dia	56.5 (56.7), 4.1 (4.0), 12.0 (12.4)	61

20,580–20,715 cm^{-1} (Table III) and are assigned[21] 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.[21,22] A high intensity band at 28460–29325 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic susceptibility measurements (3.8–4.2 B.M) for the solid Co (II) complexes are also indicative of three unpaired electrons per Co (II) ion suggesting[23] consistency with their octahedral environment.

The electronic spectra of the Cu(II) complexes (Table III) showed two low-energy weak bands at 15,115–15,235 cm^{-1} and 19,420–19,540 cm^{-1} and a strong high-energy band at 30,240–30,425 cm^{-1}. The low-energy bands in this position typically are expected for a square-planar configuration and may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively[24]. The strong high-energy band, in turn, is assigned to metal → ligand charge transfer. Also, the magnetic moment values (1.4–1.8 B.M) (Table II) for the copper (II) are indicative of an anti-ferromagnetic spin–spin interaction through molecular association. Hence, the copper (II) complexes appear to be in square-planar geometry with $d_x^2 - d_y^2$ ground state.[25]

The electronic spectra of the Ni(II) complexes showed d-d bands in the region 26,385–26,555, 15,665–15,770 and 10,260–10,315 cm^{-1}. These are assigned[26] 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 29,885–30,215 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements (3.1–3.4 B.M) showed two unpaired electrons per Ni(II) ion suggesting[27] also an octahedral geometry for the Ni (II) complexes. The electronic spectra of the Zn (II)

TABLE III Spectral Data of the Metal Chelates **1**–**20**

No	IR (cm^{-1})	λ_{max} (cm^{-1})
1	1760, 1665, 1610, 1595,415, 560	7,635, 17,350, 20,580, 28,460
2	1760, 1665, 1615, 1595, 430, 545	7,715, 17,350, 20,715, 29,325
3	1765, 1665, 1610, 1595,415, 555, 355	7,570, 17,385, 20,645, 29,275
4	1760, 1665, 1615, 1595, 1580, 425, 550	7,610,17,450, 20,685, 28,890
5	1760, 1665, 1610, 1595, 415, 560	7,585,17,415, 20,615, 28,765
6	1760, 1665, 1610, 1595, 430, 545	15,115, 19,475, 30,240
7	1760, 1665, 1615, 1595,415, 550	15,235, 19,540, 30,425
8	1760, 1665, 1610, 1595,425, 555	15,170, 19,495, 30,335
9	1760, 1665, 1615, 1595,420, 545	15,225, 19,420, 30,415
10	1760, 1665, 1610, 1595,425, 560	15,185, 19,535, 30,290
11	1760, 1665, 1610, 1595, 415, 560	10,260, 15,665, 26,385, 29,885
12	1760, 1665, 1610, 1595, 425, 545, 340	10,315, 15,715, 26,555, 30,210
13	1760, 1665, 1610, 1595, 430, 555	10,285, 15,680, 26,445, 30,215
14	1760, 1665, 1610, 1595, 1580, 420, 560	10,310, 15,710, 26,465, 29,995
15	1760, 1665, 1610, 1595, 425, 560	10,275, 15,770, 26,550, 30,190
16	1760, 1665, 1610, 1595, 415, 545	28,350
17	1760, 1665, 1610, 1595,430, 555, 340	29,145
18	1760, 1665, 1610, 1595,425, 550	28,770
19	1760, 1665, 1610, 1595, 1580, 420, 560	28,935
20	1760, 1665, 1610, 1595,420, 545	28,815

TABLE IV Antibacterial activity data for compounds **L**–**L**^{5} and **1**–**20**

Compound	Diameter of zones showing complete inhibition of growth (mm)*			
	E. coli	*P. aeruginosa*	*K. pneumoniae*	*S. aureus*
L	20	10	22	18
L^1	23	13	26	22
L^2	24	12	26	20
L^3	23	13	26	21
L^4	24	12	25	22
L^5	23	10	24	20
1	26	15	29	24
2	28	13	30	23
3	26	24	30	23
4	27	13	27	25
5	27	11	28	23
6	27	15	31	24
7	28	13	30	22
8	26	14	31	23
9	28	11	29	24
10	26	10	27	27
11	25	13	29	24
12	27	12	29	22
13	26	14	30	23
14	27	12	29	24
15	26	10	27	22
16	27	14	31	25
17	28	13	32	23
18	28	14	32	24
19	28	13	30	25
20	26	11	34	22

* >14 mm = significant activity; 7–13 mm = moderate activity; <7 mm = weak activity.[15]

complexes exhibited only a high-intensity band at 28,350–29,145 cm^{-1} and are assigned[28] to a ligand-metal charge transfer.

Biological Activity

The antibacterial activity results presented in Table IV show clearly that the newly synthesized compounds (**L**^{1}–**L**^{5}) and their metal complexes (**1**–**20**) containing Co(II), Cu(II), Ni(II) and Zn(II) possess good biological activity. The new derivatives (**L**^{1}–**L**^{5}) obtained from cephalexin sodium salt (**L**) by condensation with different heterocyclic and aromatic aldehydes, screened for their antibacterial effect against *E. coli*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* exhibited a markedly enhancement of antibiotic activity against all the test bacterial strains compared to the parent antibiotic cephalexin sodium salt (**L**). This enhancement in the activity of derivatives (**L**^{1}–**L**^{5}) may be rationalized on the basis of their structures, since **L**^{1}–**L**^{5} possess an additional C=N bond with an heterocyclic or aromatic ring. It has been suggested that ligands with nitrogen and oxygen donor systems might inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be more susceptible to deactivation by metal ions upon chelation. Chelation reduces the polarity[29,30] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups and possibly the π-electron delocalization[31,32] occuring within the whole chelate ring system formed during coordination. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn favors its permeation through the lipoid layer of the membrane.[33–37] It has also been observed that some compounds containing groups such as azomethine or heteroaromatic systems introduced into such novel ligands, exhibit extensive biological activities that may be due to the increase in hydrophobic character and liposolubility of the resulting molecules in crossing the cell membrane of the microorganism with enhancement of the biological potency and activity of the drug.

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