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Preparation, characterization and biological evaluation of copper(II) and zinc(II) complexes with Schiff bases derived from amoxicillin and cephalexin

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Copper(II) and zinc(II) complexes of Schiff bases obtained by condensation of amoxicillin and cephalexin with salicylaldehyde/pyridoxal were prepared and characterized by microanalytical, thermogravimetric, magnetic and spectroscopic data. All the complexes were found to be six-coordinate and containing two water molecules. The electron paramagnetic resonance spectral lines exhibited rhombic distortion from axial symmetry, with $g_{\parallel} > g_{\perp} > g_e$, in the copper(II) complexes. The geometry of the zinc(II) complexes appears to be octahedral. All the compounds under investigation showed antibacterial activity. The antibacterial activity showed the following trend: copper(II) complexes > zinc(II) complexes > Schiff base ligands > parent drugs. The copper(II) complexes with the Schiff bases derived from cephalexin showed substantially enhanced activity against *Pseudomonas aeruginosa* compared with the parent drug. All the copper complexes were also found to be active against kaolin paw oedema, whereas the parent drugs were inactive. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: copper complexes; zinc complexes; antibacterials; Schiff base complexes; cephalosporins

INTRODUCTION

The wide use of antibiotics in man and animals and their extensive use in areas other than the treatment and prophylaxis of disease have resulted in a serious problem of drug resistance. More and more bacterial strains have become resistant to the available drugs. Various strategies have been worked out and tried to cope with the resistance problem and enhance the activity, or broaden the spectrum of the drugs.[1] Preparation of different synthetic derivatives of antibiotics based on structure–activity relationships has been one of the best approaches. In this study we have attempted to widen the scope of derivatization by providing more flexibility through Schiff base formation with the drug substances containing $-NH_2$ groups and complexation with metal ions. The Schiff base structures provide for a greater choice and flexibility, and complexation with metal ions adds to the stability and versatility of the molecule. The drug molecules used in the present study contain $-NH_2$, –COOH and other donor groups; construction of molecular models indicates that the structures are suitable for chelate formation.

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MATERIALS AND METHODS

Materials

Amoxicillin trihydrate (Pharmagen Beximco, Pakistan), cephalexin sodium (Dobfer, Italy), and all other chemicals (E. Merck, Germany) were used without further purification.

Preparation of Schiff base ligands

Two series of Schiff base ligands were prepared by reacting the drug substances with salicylaldehyde and pyridoxal hydrochloride separately. The method of preparation was as follows. The drug substance (2 mmol) dissolved in methanol (25 cm^3) was mixed with salicylaldehyde or pyridoxal hydrochloride (2 mmol) dissolved in methanol (25 cm^3). To this, KOH (0.1% in methanol) was added to adjust the solution

Table 1. Physical properties and microanalytical data of the Schiff base ligands and their metal complexes

Comp. no.	Compound	Colour	Decomposition point (°C)	Elemental analysis (%) Found (calc.)			
				C	H	N	M
I	Salicylideneamoxycillin (M^+: 469 *m/e*)	Orange	194	58.07 (58.84)	5.13 (4.90)	8.76 (8.95)	—
II	Salicylidenecephalexin (M^+: 451 *m/e*)	Yellowish-orange	193	60.84 (61.19)	4.92 (4.65)	9.19 (9.31)	—
III	Pyridoxylideneamoxycillin (M^+: 550 *m/e*)	Dark orange	190	52.93 (52.31)	4.35 (4.72)	10.09 (10.17)	—
IV	Pyridoxylidenecephalexin (M^+: 532 *m/e*)	Dark yellow	188	54.42 (54.08)	4.10 (4.50)	10.15 (10.51)	—
V	Salicylideneamoxycillin–Cu(II) · $2H_2O$ (M^+: 1035 *m/e*)	Dark brown	196	53.11 (53.29)	4.41 (4.66)	8.02 (8.10)	6.23 (6.12)
VI	Salicylideneamoxycillin–Zn(II) · $2H_2O$ (M^+: 1037 *m/e*)	Light yellow	200	53.02 (53.20)	4.43 (4.65)	7.96 (8.09)	6.22 (6.29)
VII	Salicylidenecephalexin–Cu(II) · $2H_2O$ (M^+: 999 *m/e*)	Brown	290	54.93 (55.21)	4.50 (4.43)	8.16 (8.40)	6.41 (6.35)
VIII	Salicylidenecephalexin–Zn(II) · $2H_2O$ (M^+: 1001 *m/e*)	Light yellow	261	54.93 (55.11)	4.46 (4.42)	8.26 (8.38)	6.46 (6.52)
IX	Pyridoxylideneamoxycillin–Cu(II) · $2H_2O$ (M^+: 1197 *m/e*)	Brown	200	50.97 (51.16)	4.67 (4.83)	9.54 (9.94)	5.30 (5.63)
X	Pyridoxylideneamoxycillin–Zn(II) · $2H_2O$ (M^+: 1199 *m/e*)	Light yellow	210	50.93 (51.08)	4.72 (4.82)	9.71 (9.92)	5.38 (5.79)
XI	Pyridoxylidenecephalexin–Cu(II) · $2H_2O$ (M^+: 1161 *m/e*)	Dark yellow	270	52.68 (52.85)	4.57 (4.62)	9.89 (10.27)	5.69 (5.82)
XII	Pyridoxylidenecephalexin–Zn(II) · $2H_2O$ (M^+: 1163 *m/e*)	Light yellow	261	52.71 (52.76)	4.51 (4.61)	10.12 (10.25)	5.81 (5.98)

between pH 7 and 8 and the mixture was refluxed for 30 min (approximately). A clear, coloured solution was obtained. The Schiff base ligand was isolated by crystallization after volume reduction by evaporation. The crystalline product was dried under vacuum and kept in a desiccator till further use. The compounds thus obtained are listed in Table 1.

Preparation of Schiff base metal complexes

The Schiff base ligand (2 mmol) dissolved in methanol (25 cm^3) was mixed with $Cu(CH_3COO)_2 \cdot H_2O$(1 mmol) or $Zn(CH_3COO)_2 \cdot 2H_2O$ (1 mmol) dissolved in methanol (25 cm^3). The reaction mixture was refluxed for 2–3 h. The product was isolated after reduction of volume by evaporation. It was filtered off, washed with methanol and dried under vacuum. The complexes thus obtained are listed in Table 1.

Characterization

Microanalysis was performed by the usual techniques. Copper and zinc were estimated by atomic absorption spectrometry. Molecular masses were determined mass spectrometrically. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed with a Netzsch simultaneous thermal analyser. Conductivity measurements were made with the Orion Model 160 conductivity meter using dimethylformamide (DMF) as solvent at room temperature. The magnetic moments of the copper complexes were determined by Gouy's technique using mercury(II) tetrathiocyanatocobaltate as calibrant; diamagnetic corrections were calculated from Pascal's constants.[2] IR spectra were recorded with an FT-IR (Midac) spectrophotometer using KBr disc and Nujol mull techniques. Electronic absorption spectra were obtained with a Hitachi Model 121-0032 spectrophotometer using methanol (for the ligands) and DMF (for the complexes) as solvents. Electron paramagnetic resonance (EPR) spectra were recorded as a powder and in solution (DMF), at room temperature, on a Jeol JES-FE 1XG instrument, in the X-band, operating at a microwave frequency of 9.44 GHz. The g values were determined by use of the Kneubühl approximation.[3] The spectra were calibrated using the α,α-diphenyl-*p*-picrylhydrazyl radical ($g = 2.0036$) as a field marker. Proton NMR spectra of the zinc complexes were recorded on a 90 MHz Perkin Elmer machine using deuterated dimethylsulfoxide (DMSO-d_6) as the solvent and tetramethylsilane (TMS) as the standard.

Antibacterial activity

The antibacterial study of the complexes under investigation was performed using standard strains of *Staphylococcus aureus*

(ATCC 6538), *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027). Minimum inhibitory concentrations (MICs) were determined by the standard dilution technique[4] by use of tryptic soy broth (Difco), in which the complexes dissolved. The tubes were incubated at 37 °C for 24 h.

Anti-inflammatory activity

Kaolin paw oedema was induced, by a method reported elsewhere,[5] in male Wistar rats, 105–115 g, in groups of five. The complexes under investigation were administered orally in 5% mulgophen (GAF, Manchester) in distilled water (0.2 ml per 100 g) 1 h before the kaolin. The rats were dosed on a weight of drug (milligrams) per body weight (kilograms) of animal basis. Oedema was evaluated 4 h after sub-plantar administration of kaolin in 0.9% w/v sodium chloride solution. Inhibition of oedema was evaluated by comparing the swelling obtained in treated animals with that in controls and was expressed as percentage inhibition. Statistical significance was evaluated by use of the Student *t*-test.

Toxicity study

Experiments were performed on albino Wistar male rats, 160–180 g. Animals were kept at constant temperature (25 ± 0.5 °C) and humidity. Conventional laboratory diet and water were freely available. The complexes under investigation were administered orally in 0.15% agar suspension (50 $cm^3\ kg^{-1}$) to four groups of 10 rats. After treatment, the animals were monitored every hour for several hours and then every day for 14 days.

RESULTS AND DISCUSSION

The complexes under investigation were isolated by refluxing the copper(II) acetate monohydrate and zinc(II) acetate dihydrate with Schiff base ligands in methanol. The isolated complexes were fairly soluble in DMF and DMSO and insoluble in other common organic solvents. The complexes were found to be hygroscopic.

Microanalytical data (Table 1) confirmed the ML_2 composition of the complexes, in which M is copper(II) or zinc(II) and L is the Schiff base ligand. The complexes decomposed between 196 to 290 °C and did not show sharp melting points.

Karl–Fischer titration and thermal analysis (TGA and DTA) indicated the presence of water molecules in the complexes. There was a weight loss equivalent to two water molecules in all the complexes around 120 °C, showing that the water is coordinated. From 196 to 500 °C, a sharp decrease in weight indicated a loss of one of the Schiff base ligands from the complexes. Thus, the thermal decomposition of complexes may be represented by the following equations:

$$ML_2 \cdot xH_2O \longrightarrow ML_2 + xH_2O$$

$$ML_2 \longrightarrow ML + L$$

The molecular masses determined mass spectrometrically (Table 1) also confirmed the ML_2 composition.

The bonding of the ligands to metal ions was investigated by comparing the FT-IR spectra of the complexes with those of the free ligands. The FT-IR spectra of the complexes contained all the absorption bands from the ligands and some new absorption bands indicative of coordination of the ligands with metal ions through nitrogen and oxygen. Some important absorption bands and their assignments are listed in Table 2.

The spectra of these complexes exhibited a broad band around 3395–3500 cm^{-1}, which is assigned to water molecules, ν(OH), associated with the complexes. In addition to these modes, coordinated water exhibited $\rho_r(H_2O)$ rocking near 885, 845 cm^{-1} and $\rho_w(H_2O)$ wagging near 540 cm^{-1}.[6] FT-IR spectra of all the ligands contained a band at 1610–1630 cm^{-1}, ν(C=N), which shifted slightly to a higher value in all the complexes suggesting that the ligands are coordinated to the metal ion through –C=N–.[7] The absorption due to the carboxylic group did not change in

Table 2. Observed IR frequencies (cm^{-1}) and assignments

Comp. No.	ν(OH)	ν(C=N)	$\rho_r(H_2O)$	$\rho_w(H_2O)$	δ(CO)	π(CO)	ν(MN)	ν(MO)
I	3540	1615	—	—	750	560	—	—
II	3500	1610	—	—	740	575	—	—
III	3510	1620	—	—	745	570	—	—
IV	3515	1630	—	—	740	580	—	—
V	3480	1630	885, 840	540	750	555	440	335
VI	3490	1635	880, 845	535	755	560	435	340
VII	3472	1630	885, 845	545	750	580	438	335
VIII	3395	1625	890, 847	540	753	578	460	350
IX	3500	1626	885, 850	545	740	572	445	340
X	3490	1628	890, 845	540	745	575	454	350
XI	3485	1635	880, 840	535	735	575	442	335
XII	3483	1632	885, 850	540	740	578	460	355

Table 3. Physical data of the ligands and complexes

Comp. no.	Conductance (μS cm^{-1})	Geometry	μ(BM)	λ/nm (ε/cm^{-1} mol^{-1})	
				Ligand bands	d–d bands
I	16.3	—	—	220 (20 469), 240 (18 896), 350 (4284)	—
II	36.7	—	—	210 (21 760), 250 (15 756)	—
III	20.9	—	—	210 (17 617), 280 (7865)	—
IV	32.7	—	—	212 (20 226), 283 (6138)	—
V	12.5	Octahedral	2.22	220 (7000), 241 (6440), 352 (1464)	650 (635)
VI	10.1	Octahedral	Diamagnetic	220 (7012), 240 (6437), 350 (1462)	—
VII	8.3	Octahedral	2.17	210 (7444), 252 (5389)	641 (629)
VIII	10.9	Octahedral	Diamagnetic	210 (7438), 251 (5365)	—
IX	18.9	Octahedral	2.32	211 (5948), 283 (2655)	653 (690)
X	17.7	Octahedral	Diamagnetic	210 (5942), 281 (2650)	—
XI	14.6	Octahedral	2.21	214 (6904), 284 (2095)	655 (711)
XII	17.8	Octahedral	Diamagnetic	212 (6906), 282 (2090)	—

the spectra of the complexes, indicating that the carboxylic groups are not involved in coordination with the metal ion. New absorption bands ν(MN) and ν(MO) appeared at 435–460 cm^{-1} and 335–355 cm^{-1} respectively in the spectra of the complexes, indicating coordination of the ligands through nitrogen and oxygen.

In the electronic absorption spectra of the complexes (Table 3) there is an intense band at 240–284 nm, which is assigned to a π–π^* transition originating in the phenyl ring. The low-energy broad absorption band in the range 641–655 nm in the spectra of the copper complexes arises from a d–d transition.

Formation of the Schiff base was confirmed by extinction of the aldehyde proton signal at 9.2 ppm and the appearance of the aldimine proton signal at 7.6 ppm with reference to TMS in the ^{1}H NMR spectra. The coordination of an imino group to the metal ion was confirmed by a change in chemical shift of the aldimine proton to 7.3 ppm on complexation with zinc. Lower molar conductance values (8.3–18.9 μS cm^{-1}) for the complexes (Table 3) indicate the non-electrolytic nature of the complexes.

The copper complexes had normal values of the magnetic moments, i.e. 2.17–2.32 BM (Table 3), indicating their mononuclear nature. The EPR parameters of the copper complexes are given in Table 4. There was a general correspondence between the powder and solution spectra; however, the $g_\parallel$ region was well resolved in solution. The spectra were indicative of rhombic distortion from axial symmetry. Owing to coordination of two different kinds of atoms (oxygen and nitrogen) with the metal ion, the spectra were characteristic of magnetically dilute systems with copper(II) ions in the $d_{x^2-y^2}$ ground state ($g_\parallel > g_\perp > g_e$). Hyperfines as a result of nitrogen ($I = 1$) were visible on the main absorption line $g_\perp$, confirming the coordination through nitrogen.

Table 4. EPR parameters of the copper complexes

Comp. no.	Solid		Solution		
	$g_{\|\|}$	$g_\perp$	$g_{\|\|}$	$g_\perp$	$\|A_{\|\|(Cu)}\|$
V	2.15	2.06	2.261	2.020	16.21
VII	2.18	2.07	2.285	2.024	16.48
IX	2.19	2.04	2.300	2.021	16.20
XI	2.20	2.05	2.260	2.028	16.00

Based on the experimental evidence thus obtained, the complexes were characterized as six-coordinates with the two positions occupied by two water molecules. Zinc is known to form four-, five- or six-coordinate complexes. The coordination number six is favoured when oxygen donors are present. In the complexes under investigation there are two water molecules, which occupy the fifth and sixth positions in the octahedron. The hydrated complexes have significance in the enzymatic systems, as the substrates can bind to zinc by substituting the coordinated water molecules in the complexes. Six-coordinate complexes of zinc(II) derived from indomethacin[8] and other ligands[9,10] have also been reported.

The proposed structures of the complexes under investigation, on the basis of the above experimental evidence, are shown in Figures 1–4. Unsuccessful attempts to isolate crystals suitable for X-ray analysis prevented further structure elucidation.

Antibacterial activity

The results of antibacterial study are given in Table 5. A cursory view of the data indicates the following trend in activity of the substances under investigation against *E. coli* and *S. aureus*:

copper(II) complexes > zinc(II) complexes

> Schiff base ligands > parent drugs

The copper(II) complexes were found to be 11–17 times more active than the parent drugs, 8–9 times more active than

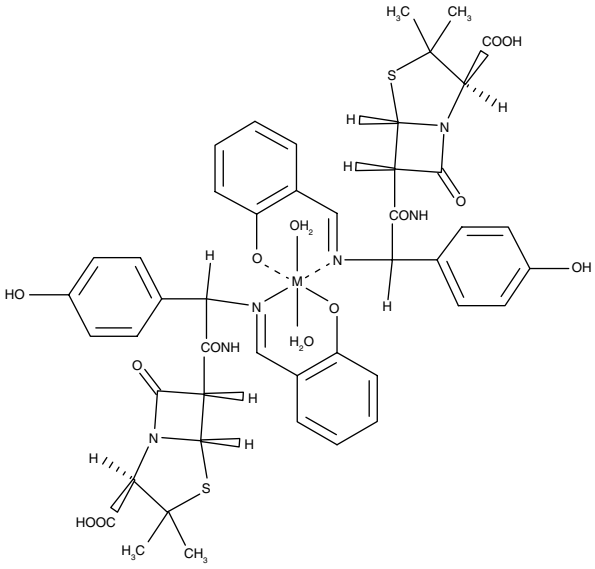

Figure 1. Proposed structure of metal complexes of salicylideneamoxicillin. M: copper(II) or zinc(II).

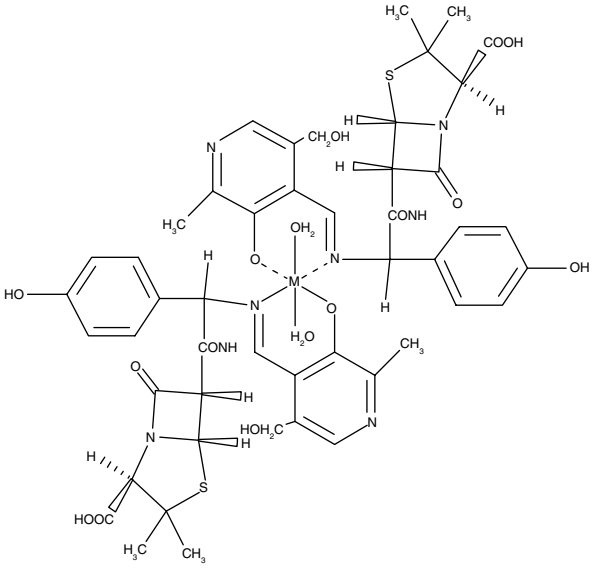

Figure 3. Proposed structure of metal complexes of pyridoxylideneamoxicillin. M: copper(II) or zinc(II).

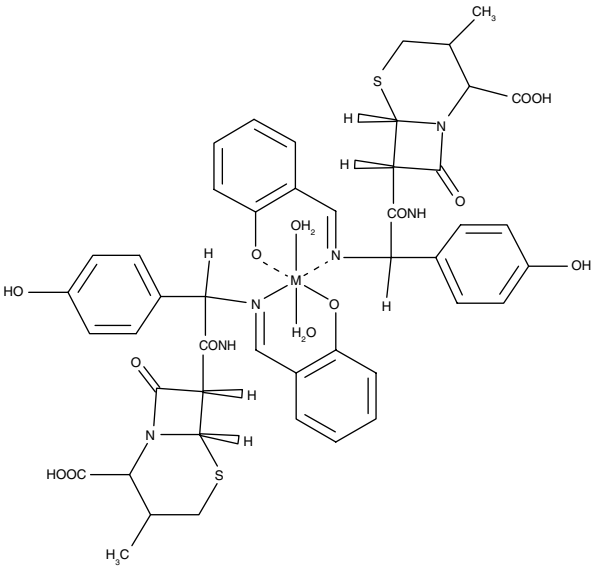

Figure 2. Proposed structure of metal complexes of salicylidenecephalexin. M: copper(II) or zinc(II).

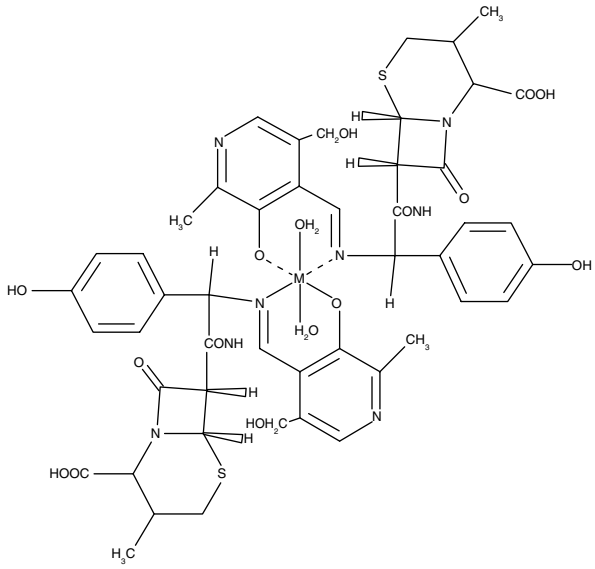

Figure 4. Proposed structure of metal complexes of pyridoxylidenecephalexin. M: copper(II) or zinc(II).

the Schiff base ligands, and slightly more active than the zinc complexes against *E. coli*. In contrast, the copper(II) complexes were found to be 2–4 times more active than the parent drugs, 1.6–2.3 times more active than the Schiff base ligands, and up to 1.5 times more active than the zinc complexes against *S. aureus*. All the Schiff base ligands under study were 1.2–2 times more active than the parent drugs against *E. coli* and *S. aureus*. The copper(II) complexes showed substantially enhanced activity against *P. aeruginosa* compared with the parent drug. The enhanced activity of the complexes may be attributed to the facilitation provided by the metal ion for binding of the drug with the substrate through coordination.

Anti-inflammatory activity

The results of the paw oedema test are summarized in Table 5. The copper complexes were found to be active, whereas the zinc complexes and the parent drugs had no significant activity. The results are in line with previous findings.[11]

Table 5. MICs, LD_{50} values and anti-inflammatory activity data

Comp. no.	Dose (mg kg^{-1})	Inhibition of oedema[a] (%)	LD_{50}[b] (g kg^{-1})	MIC (μg cm^{-3})		
				E. coli	*S. aureus*	*P. aeruginosa*
Amoxicillin	50	3	2.5	96	12.00	>300
Cephalexin sodium	50	4	5.5	100	10.00	>300
I	50	4	5.0	70.0	8.50	>300
II	50	4	7.1	50.5	5.00	>300
III	50	4	10.0	77.0	9.30	>300
IV	50	5	8.0	55.0	5.50	>300
V	50	40	4.7	8.5	5.00	>200
VI	50	6	5.2	9.0	5.50	>200
VII	50	45	6.8	5.8	2.30	>150
VIII	50	5	7.0	7.0	3.50	>200
IX	50	48	2.2	9.0	5.80	>200
X	50	6	2.4	9.8	6.00	>200
XI	50	50	5.3	6.5	2.50	>170
XII	50	4	5.5	7.5	4.00	>200

[a] All $P < 0.05$ compared with control.
[b] Quantity resulting in the death of the half the number of rats.

Toxicity study

The LD_{50} values (quantities resulting in the death of half the rats) are given in Table 5. Toxicity was reduced (lower LD_{50} values) by complexation.

CONCLUSIONS

These results show that the antibacterial activity and toxicity of the drugs under investigation is improved after derivatization. The drugs acquired anti-inflammatory activity on complexation with copper. These observations, in line with other studies, suggest that the metal-based drugs possess great potential as therapeutics.

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