

Metalloantibiotics: Synthesis and Antibacterial Activity of Cobalt(II), Copper(II), Nickel(II) and Zinc(II) Complexes of Kefzol

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Kefzol (kzl), a β -lactam antibiotic, possesses various donor sites for interaction with transition metal(II) ions [Co(II), Cu(II), Ni(II) and Zn(II)] to form complexes of the type $[M(kzl)_2]Cl_2$ and $[M(kzl)Cl]$, with molar ratio of metal: ligand (M:L) of 1:2 and 1:1 respectively. These complexes were prepared and characterized by physico-chemical and spectroscopic methods. Their IR and NMR spectra suggest that kefzol potentially acts as a bidentate, tridentate as well as monoanionic tetradentate ligand. The complexes have been screened for antibacterial activity and results were compared with the activity of the uncomplexed antibiotic against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*. The metal complexes were found to be more potent against one or more bacterial species than the uncomplexed kefzol.

Keywords: Kefzol; Metal complexes; Antibacterials

INTRODUCTION

Metalloorganic chemistry is becoming an emerging area of research due to the demand for new metal-based antibacterial compounds. The serious medical problem of bacterial resistance and the rate at which it develops have led to increasing levels of resistance to classical antibiotics among Gram positive organisms such as *Pneumococci*, *Enterococci* and *Staphylococci*. The actual mechanism involved is the release of relatively large amounts of β -lactamase into the surrounding medium and thus the destroying of the β -lactamic antibiotics by hydrolysis

of the lactam ring has become the most predominant mechanism of resistance.^{1–3}

Several *in vivo* studies have indicated^{4–7} that metal complexes possess interesting toxicological and pharmacological properties. However, a significant problem is that some of them lose their activity upon exposure to proteins, and appear to have better affinities than the ligands/compounds studied for metal ions, which are deactivated once again when embedded in the proteins. We have previously investigated^{8–15} the coordination chemistry of some antibiotics and/or antibacterial compounds with transition metals to examine the modes of binding and to study their effect on biological activity. Continuing our investigations on the interaction of metal ions with antibiotics, we report here the synthesis and characterization of kefzol (Figure 1) metal complexes and their antibacterial properties against various pathogenic bacterial strains of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*.

MATERIAL AND METHODS

Kefzol sodium salt was obtained from Pharmagen Beximco Ltd, Pakistan. Solvents used were analar grade. All metal(II) salts were used as chlorides. IR spectra (KBr pellets) were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer and NMR spectra were recorded on a Perkin-Elmer 283B spectrometer. UV-Visible spectra were obtained in

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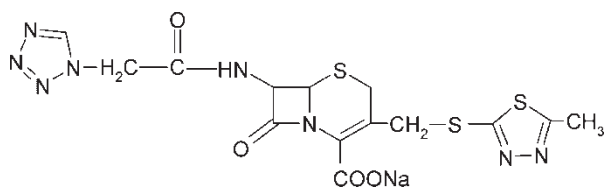


FIGURE 1 Structure of Kefzol sodium salt

DMF on a Hitachi U-2000 double-beam spectrophotometer. C, H and N analyses were carried out by Butterworth Laboratories Ltd (U.K). Conductances of the metal complexes were determined in DMF on a Hitachi YSI-32 model conductometer. Magnetic measurements were done on solid complexes using the Gouy's method. Melting points were recorded on a Gallenkamp apparatus and are uncorrected.

Preparation of Metal(II) Complexes

The complex, $[M(kzl)Cl]$ was prepared by mixing kefzol (2 mmol) and metal(II) as chlorides (2 mmol) in methanol (50 mL) and $[M(kzl)_2]Cl_2$, by mixing kefzol (2 mmol) and metal(II) chloride (1 mmol) in methanol (50 mL). The pH of the solution was adjusted to 8.0 with 5.0 M NaOH and the mixture was refluxed for 1 h and then cooled at room temperature. On cooling a solid metal complex was precipitated which was filtered off, washed with methanol, then with ether and dried. Crystallization from aqueous-methanol (30:70) gave the desired metal complexes.

Biological Activity

The antibacterial activities of kefzol and all its synthesized metal(II) chelates were screened

in vitro using the disc diffusion method.^{16–20} The chosen strains, *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739) (incubation period at 37°C for 24 h), *Pseudomonas aeruginosa* (ATCC 10145), *Klebsiella pneumoniae* (ATCC 556) (incubation period at 37°C for 24 h) and *Proteus mirabilis* (ATCC 497) were obtained from the Microbiology Laboratory, Qaid-e-Azam Medical College, Bahawalpur, Pakistan. Test solutions of kefzol and its metal(II) complexes were prepared in DMF. The bacteria were cultured for 24 h at 37°C in an incubator. Muller Hinton broth was used for preparing basal media for the bioassay of the organisms. Nutrient agar was poured onto a plate and allowed to solidify. The test compounds solutions (DMF) were added dropwise to a 10 mm diameter filter paper disc placed at the centre of each agar plate. The plates were then kept at 5°C for 1 h then transferred to an incubator maintained at 37°C. The width of the growth inhibition zone around the disc was measured after 24 h incubation.

RESULTS AND DISCUSSION

Chemistry

The structures of the new compounds reported herein were established with the help of their IR, H NMR and microanalytical data. The elemental analyses data agree well with the proposed formulae for the complexes (Table I). Cobalt(II) and copper(II) complexes were pink and green respectively, while the nickel(II) and zinc(II) were dark green and off-white, respectively. All metal complexes (1–8) are air and moisture stable solids and were prepared by the stoichiometric reaction of the corresponding metal(II) chloride with the kefzol, in a molar ratio

TABLE I Physical and Analytical Data of the Metal(II) Complexes

Metal chelate/Mol.weight/Mol. Formula	M.P (°C)	B.M (μ_{eff})	Calc. (Found) %			Yield (%)
			C	H	N	
1 $[Co(kzl)_2]Cl_2$ [1038.2] $C_{28}H_{28}N_{16}CoCl_2O_8S_6$	221–223	3.8	32.4 (32.9)	2.7 (2.4)	21.6 (21.2)	56
2 $[Cu(kzl)_2]Cl_2$ [1042.8] $C_{28}H_{28}N_{16}CuCl_2O_8S_6$	225–227	1.8	32.2 (32.0)	2.7 (2.8)	21.5 (21.3)	61
3 $[Ni(kzl)_2]Cl_2$ [1038.0] $C_{28}H_{28}N_{16}NiCl_2O_8S_6$	223–225	3.2	32.4 (32.1)	2.7 (2.9)	21.6 (21.3)	57
4 $[Zn(kzl)_2]Cl_2$ [1044.7] $C_{28}H_{28}N_{16}ZnCl_2O_8S_6$	217–218	Dia	32.2 (32.6)	2.7 (2.4)	21.4 (21.6)	56
5 $[Co(kzl)Cl]$ [548.6] $C_{14}H_{14}N_8CoClO_4S_3$	189–191	3.9	30.6 (30.9)	2.6 (2.8)	20.4 (20.1)	58
6 $[Cu(kzl)Cl]$ [553.2] $C_{14}H_{14}N_8CuClO_4S_3$	195–197	1.6	30.4 (30.8)	2.5 (2.9)	20.2 (20.0)	63
7 $[Ni(kzl)Cl]$ [548.4] $C_{14}H_{14}N_8NiClO_4S_3$	202–204	3.1	30.6 (30.4)	2.6 (2.3)	20.4 (20.6)	60
8 $[Zn(kzl)Cl]$ [555.1] $C_{14}H_{14}N_8ZnClO_4S_3$	190–192	Dia	30.3 (30.5)	2.5 (2.1)	20.2 (20.5)	58

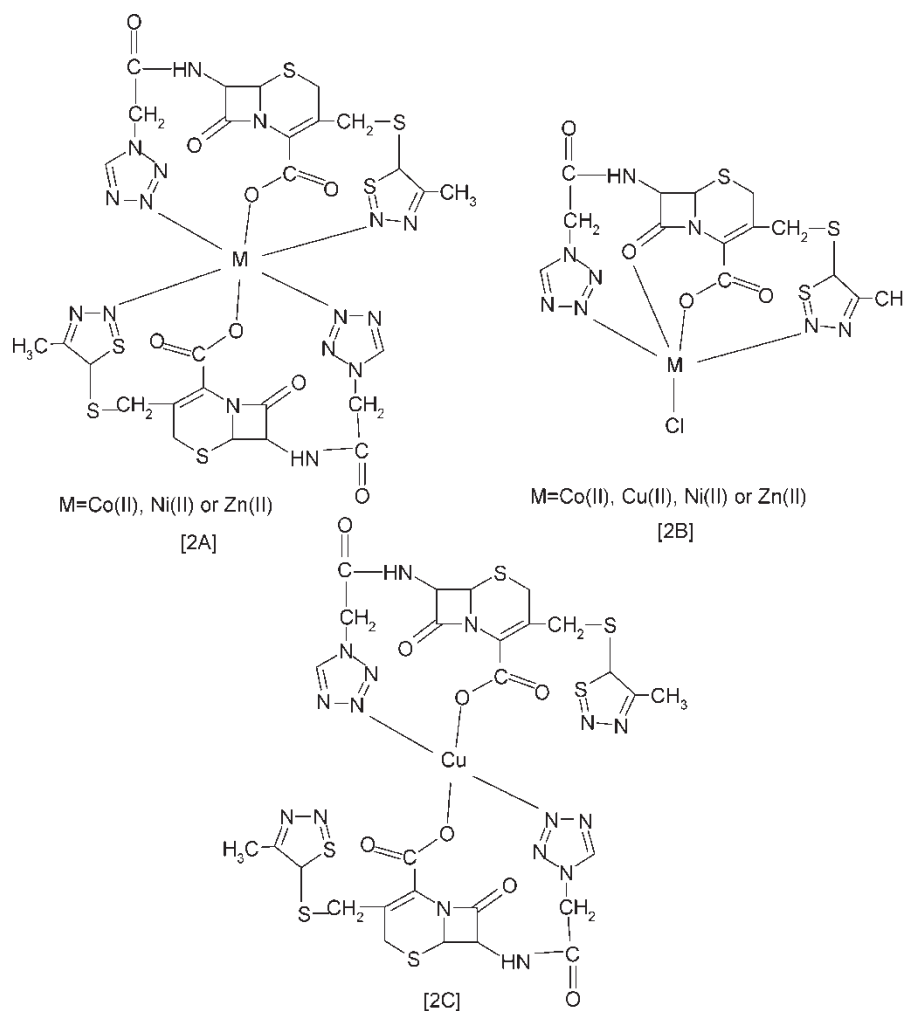


FIGURE 2 Proposed Structure of the Metal Complexes of Kefzol

M:L of 1:1 and 1:2 (Figure 2). These complexes are only soluble in DMSO, DMF and water and insoluble in methanol and ethanol. The conductivity values, measured in DMF at room temperature, fall within the range 76–85 and 12–16 $\text{ohm}^{-1} \text{cm}^{-2} \text{mol}^{-1}$ suggesting²¹ the $[\text{M}(\text{kzl})_2]\text{Cl}_2$ complex to be all electrolytic and the $[\text{M}(\text{kzl})\text{Cl}]$, complex to be non-electrolytic in nature,¹⁹ respectively. Efforts to grow good crystals for X-ray diffraction studies were

unsuccessful due to their poor solubility in common organic solvents.

IR Spectra

The IR spectra of kefzol and its complexes are reported in Table II. The lactam $\nu(\text{C}=\text{O})$ band appears at 1750 cm^{-1} in the spectrum of kefzol while the amide $\nu(\text{C}=\text{O})$ band appears at 1645 cm^{-1} ;

TABLE II Spectral Data of the Metal Chelates

No	IR (cm^{-1})	λ_{max} (cm^{-1})
Kefzol	1765, 1750, 1645, 1485, 1110, 955	–
1	1750, 1645, 1610, 1595, 560, 465	7,635, 17,350, 20,580, 28,460
2	1750, 1645, 1610, 1595, 545, 475	15,115, 19,475, 30,240
3	1750, 1645, 1610, 1595, 555, 475	10,275, 15,770, 26,550, 30,190
4	1750, 1645, 1610, 1595, 550, 465	28,350
5	1735, 1645, 1610, 1595, 560, 415, 375	7215, 16190, 29335
6	1735, 1645, 1610, 1595, 545, 430, 375	14225, 17190, 29115
7	1730, 1645, 1610, 1595, 555, 425, 375	25225, 10110, 30115
8	1745, 1645, 1610, 1595, 560, 415, 375	26275

the complexes having a molar ratio of metal: kefzol as 1:2 exhibit unchanged amide and lactam bands. These observations suggest that kefzol is not coordinated to the metal ions through amide or lactam oxygens. The carboxylate ligand can bind to a metal atom either as a monodentate or bidentate ligand, causing changes in the relative positions of the asymmetric and symmetric vibrations.²² The spectra of the complexes exhibit a separation of $>200\text{ cm}^{-1}$ suggesting monodentate bonding for the carboxylate group of kefzol with the metal ion. A new band at 1585 cm^{-1} observed in the spectra of the complexes furthermore, suggested²³ bonding of the carboxylate $\nu(\text{M-OOC})$ group. The presence of strong $\nu(\text{M-N})$ and $\nu(\text{M-O})$ stretching vibrations at $465\text{--}475\text{ cm}^{-1}$ and $545\text{--}560\text{ cm}^{-1}$ for the metal complexes (absent in kefzol), support coordination of kefzol through heteroaromatic moieties, thiadiazole and tetrazole ring nitrogens and carboxylate and, lactam oxygen. In the complexes, $[\text{M}(\text{kzl})\text{Cl}]$ (5–8), a low frequency band at 375 cm^{-1} assigned to $\nu\text{M-Cl}$ was observed. Moreover, the lactam band at 1750 cm^{-1} moved to lower frequency by $15\text{--}20\text{ cm}^{-1}$ indicating²⁴ involvement/coordination of the lactam oxygen to the metal ion. The coordination chemistry of some β -lactam antibiotics with transition and d^{10} metal ions has been reported. In the present studies, the kefzol molecule has several potential donor atoms but, due to steric constraints, the molecule can provide a maximum of three or four donor sites to the metal atom. The assumption that the kefzol molecule coordinates through the carboxylate, lactam ring and/or heteroaromatic nitrogen of the thiadiazole and tetrazole seems likely from molecular model studies. In the complexes, $[\text{M}(\text{kzl})_2]\text{Cl}_2$ where $\text{M} = \text{Co}(\text{II}), \text{Ni}(\text{II})$ or $\text{Zn}(\text{II})$ (1–4), kefzol acts as tridentate and hence forms an octahedral geometry (Figure 2A). It is quite likely that the metal ions in the $[\text{M}(\text{kzl})\text{Cl}]$ complexes where $\text{M} = \text{Co}(\text{II}), \text{Cu}(\text{II}), \text{Ni}(\text{II})$ or $\text{Zn}(\text{II})$ are pentacoordinate, with one molecule of kefzol and the chloride anion probably having tetragonal pyramidal or trigonal bipyramidal geometries (Figure 2B).²⁴ The IR spectrum of the $[\text{Cu}(\text{kzl})_2]\text{Cl}_2$ complex showed neither involvement of the lactam group nor the amide moiety suggesting²⁴ its square-planar geometry (Figure 2C).

The ^1H NMR spectrum of the diamagnetic zinc(II) complex showed strong evidence for coordination of the COO moiety to the metal ions. Normally there is a signal at 10.8 ppm assigned to the COOH proton of kefzol but this is absent in its zinc(II) metal complex suggesting formation of the metal-COO bond.

Electronic and Magnetic Properties

The UV-Visible spectra of kefzol and its complexes in DMF exhibit absorption maxima at 255–260 nm,

assigned to $\pi \rightarrow \pi^*$ transition due to molecular orbital energy levels originating in the N-C-S moiety.^{25,26} An intraligand band at 270–285 nm is related to the $\pi \rightarrow \pi^*$ transitions within the tetrazole and thiadiazole moieties. A shoulder (310–320 nm) corresponds to an intraligand $\pi \rightarrow \pi^*$ transition, attributed to a transition between energy levels originating in the S-C-S moiety. The band in the region 340–365 nm is ascribed to an intraligand transition of the $n \rightarrow \pi^*$ type in accordance with the literature data for transitions between levels due to sulfur atoms. The local symmetry around the metal(II) ions belongs to the point group C_{2v} , therefore, accurate band assignments are not possible due to the multicomponent nature of the bands.

The Co(II) complex of the type $[\text{Co}(\text{kzl})_2]\text{Cl}_2$, exhibited well-resolved, low-energy bands at $7,635\text{ cm}^{-1}$ and $17,350\text{ cm}^{-1}$ and a strong high-energy band at $20,580\text{ cm}^{-1}$ (Table II) and are assigned²⁷ to the transitions $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{2g}(\text{F})$, $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{A}_{2g}(\text{F})$ and $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{2g}(\text{P})$ for a high-spin octahedral geometry.^{28,29} A high intensity band at $28,460\text{ cm}^{-1}$ was assigned to the metal ligand charge transfer. From the molar magnetic susceptibility measurements, corrected magnetic moments were calculated using Pascals' constants. The magnitudes of the magnetic moments fall within the ranges associated with high spin ions in octahedral fields and are unlikely to be of value in discriminating between metal ions in O_h and pentagonal field symmetries. The cobalt(II) complexes have a magnetic moment of ~ 3.9 B.M typical for a high spin d^7 -system with three unpaired electrons suggesting²⁷ consistency with their octahedral environment (Figure 2A). The Co(II) complex of the type $[\text{Co}(\text{kzl})\text{Cl}]$, exhibited bands as reported in Table II which were consistent with their 5-coordinated geometry (Figure 2B).

The electronic spectra of the Cu(II) complex of the type $[\text{Cu}(\text{kzl})_2]\text{Cl}_2$ (Table II) showed two low-energy weak bands at $15,115\text{ cm}^{-1}$ and $19,475\text{ cm}^{-1}$ and a strong high-energy band at $30,240\text{ cm}^{-1}$. The low-energy bands in this position is typical for that expected for a square-planar configuration (Figure 2C) and may be assigned to $^2\text{B}_{1g} \rightarrow ^2\text{A}_{1g}$ and $^2\text{B}_{1g} \rightarrow ^2\text{E}_g$ transitions, respectively.²⁷ The strong high-energy band, in turn, is assigned³⁰ to metal \rightarrow ligand charge transfer. Also, the magnetic moment of Cu(II) complex has a value 1.8 B.M at room temperature, in the range associated with a d^9 -system with one unpaired electron. However, the electronic spectrum of the Cu(II) complex of the type $[\text{Cu}(\text{kzl})\text{Cl}]$, showed bands consistent with its 5-coordinated configuration.²⁹

The electronic spectra of the Ni(II) complex of the type $[\text{Ni}(\text{kzl})_2]\text{Cl}_2$ showed d-d bands in the region $26,550, 15,770$ and $10,275\text{ cm}^{-1}$. These are assigned³¹

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 30,210 and cm^{-1} was assigned to metal \rightarrow ligand charge transfer. All the nickel(II) complexes have a magnetic moment of ~ 3.1 B.M as predicted for high spin a d^8 -system with two unpaired electrons suggesting²⁸ also an octahedral geometry for the Ni(II) complex. The electronic spectra of the nickel complex of the type $[\text{Ni}(\text{kzl})\text{Cl}]$, showed as the expected bands suggesting³⁰ its 5-coordinated geometry. The Zn(II) complexes exhibited only a high-intensity band in the region at 28,350 and 26275cm^{-1} for $[\text{Zn}(\text{kzl})_2]\text{Cl}_2$ and $[\text{Zn}(\text{kzl})\text{Cl}]$, complexes assigned²⁸ to their ligand-metal charge transfer respectively.

BIOLOGICAL ACTIVITY

All the newly synthesized complexes (1–8) of kefzol coordinated with the metals Co (II), Cu (II), Ni (II) and Zn (II) were screened for their antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *P. mirabilis*. The metal(II) complexes exhibited a marked enhancement in kefzol activity against all the tested bacterial strains (Table III). The metal complexes with two kefzol molecules coordinated to the one central metal atom (1–4) were more antibacterial than the complexes having one kefzol molecule attached with the metal atom (5–8). This enhancement in activity may be due to an efficient diffusion of the metal complex into the bacterial cell and/or interaction with the bacterial cell. It has been further suggested that ligands having nitrogen and oxygen donor systems might inhibit those enzyme which require these groups for their activity to be especially more susceptible to deactivation by the metal ions upon chelation.

Chelation/coordination also reduces the polarity³² of the metal ion mainly because of the partial sharing

of its positive charge with these donor groups and possibly the π -electron delocalization^{41,42} within the whole chelate ring system. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favors its permeation through the lipid layer of the membrane^{33,34} thus causing the metal complex to cross the membrane of the microorganism cell wall and hence increases the bioavailability and activity of the drug.

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TABLE III Antibacterial Activity of complexes

Ligand/complexes	a	b	c	d
Kefzol sodium	18	20	10	22
1	24	26	15	29
2	23	28	13	30
3	23	26	14	30
4	25	27	13	27
5	21	23	13	26
6	20	24	11	28
7	19	22	11	27
8	22	25	12	25

Microbial species: (a) = *Staphylococcus aureus*, (b) = *Escherichia coli*, (c) = *Klebsiella pneumoniae*, (d) = *Proteus mirabilis*. Diameter of zones for complete inhibition of growth (mm). Significant activity 14–20 mm, Moderate activity 7–13 mm, Weak activity <7 mm.

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