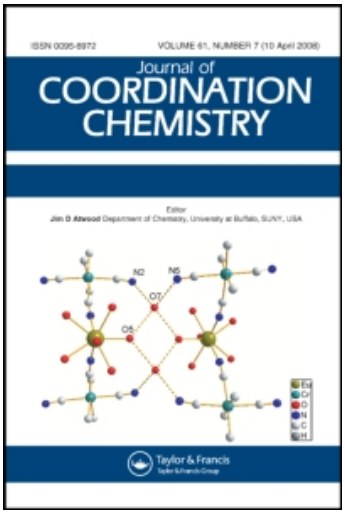

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Metalloantibiotics: synthesis and antibacterial activity of cefepime metal complexes

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Metalloantibiotics: synthesis and antibacterial activity of cefepime metal complexes

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Cefepime interacts with transition metal(II) ions to give [M(cefepime)Cl_2] complexes (M = Mn(II), Co(II), Ni(II), Cu(II), and Zn(II)) which were characterized by physicochemical and spectroscopic methods. The complexes are insoluble in water and common organic solvents, and probably have polymeric structures. The spectra indicated that the ligand is a multidentate chelating agent. The complexes have been screened for antibacterial activity against several bacteria and showed activity less than that of free cefepime.

Keywords: Cefepime metal complexes; Antibacterial activity; Transition metal complexes

1. Introduction

Cefepime is a new parenteral cephalosporin that has been described as a fourth generation broad-spectrum antibiotic [1, 2]. It is active against some bacteria that are resistant to other antibiotics and is used to treat gram-negative and gram-positive bacteria especially those causing infections in the lungs, kidneys, bladder, skin, and abdomen [3, 4].

The cephalosporin antibiotics are comprised of several different classes of compounds with dissimilar spectrums of activity and pharmacokinetic profiles [5]. They are usually bactericidal against susceptible bacteria and act by inhibiting mucopeptide synthesis in the cell wall resulting in a defective barrier and an osmotically unstable spheroplast [6]. The exact mechanism for this effect has not been determined, but β-lactam antibiotics have been shown to bind to several enzymes (carboxypeptidases, transpeptidases, and endopeptidases) within the bacterial cytoplasmic membrane that are involved with cell wall synthesis [7–10].

The different affinities that various β-lactam antibiotics have for these enzymes (also known as penicillin-binding proteins) help to explain the differences in activity of these drugs that are not explained by the influence of β-lactamases [11]. In continuation of our work on metalloantibiotics [12–18], we report here the synthesis and

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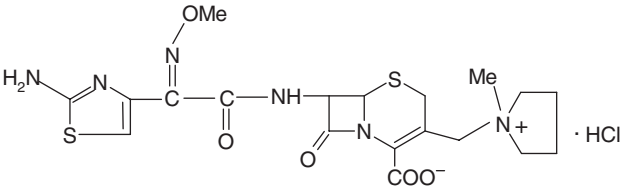

Figure 1. The structure of dipolar ion cefepime.

bactericidal activity of cefepime metal complexes. The chemical structure of cefepime is shown in figure 1.

2. Experimental

2.1. *Physical methods*

The IR spectra of the ligand and its metal complexes were recorded as KBr pellets in the 4000–400 cm^{-1} range with a Perkin–Elmer Series 2000 spectrophotometer. FTIR spectra as polyethylene pellets were registered between 450 and 120 cm^{-1} using a Bruker IFS 66V spectrophotometer. UV–Vis spectra were recorded using a Perkin–Elmer recording spectrometer. C, H, N, and S were analyzed on a LECO CHNS 932 model microanalytical instrument. Metal contents were estimated spectrophotometrically on an atomic absorption spectrometer. Differential scanning calorimetric (DSC) was measured using a Thermo DSC-Q20 instrument at a heating rate of 4°C min^{-1} up to 200°C. Magnetic susceptibilities were measured on a Johnson Matthey Susceptibility Balance at room temperature using $Hg[Co(NCS)_4]$ as calibrant. EPR spectra were recorded on a Bruker ECS 106 spectrometer by the X-band. ^{1}H-NMR was run at 80 MHz on a Varian spectrometer.

2.2. *Antibacterial activity*

In vitro antibacterial activities of cefepime and its complexes were tested using the paper disc diffusion method [19]. The chosen strains were G(+) *Staphylococcus aureus* ATCC 6538 and G(−), *Bacillus cereus* 6538, *Salmonella typhimurium* ATCC 10031, *Pseudomonas aeruginosa* ATCC 9920, and *Escherichia coli* ATCC 10536. The liquid medium containing the bacterial subcultures was autoclaved for 20 min at 121°C and at 15 lb pressure before inoculation. The bacteria were then cultured for 24 h at 36°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 in DMSO solutions were added dropwise to a 10-mm diameter filter paper disc placed at the center of each agar plate. The plates were kept at 5°C for 1 h and then transferred to an incubator maintained at 36°C. The width of the growth inhibition zone around the disc was measured after 24 h incubation. Four replicates were made for each treatment. Growth inhibition was compared with the cefepime drug; in order to clarify any participating role of DMSO and the metal(II) chloride salts

Table 1. Elemental analyses for the complexes.

Compound	Found (Calcd) %				
	C	H	N	S	Metal
[Mn(cefepime)Cl_2]	36.7 (36.6)	3.6 (3.6)	14.6 (14.2)	10.5 (10.8)	9.5 (9.3)
[Co(cefepime)Cl_2]	36.5 (36.4)	3.4 (3.5)	13.9 (14.1)	10.4 (10.8)	9.7 (9.9)
[Ni(cefepime)Cl_2]	36.7 (36.3)	3.6 (3.5)	14.3 (14.1)	10.6 (10.8)	9.5 (9.9)
[Cu(cefepime)Cl_2]	36.4 (36.0)	3.8 (3.5)	13.8 (14.0)	10.3 (10.7)	10.4 (10.6)
[Zn(cefepime)Cl_2]	35.6 (35.9)	3.4 (3.5)	14.3 (14.0)	10.9 (10.7)	10.7 (10.9)

in the biological screening, separate studies were carried out with the solutions alone of DMSO and the free metal salts and they showed no significant activity against any bacterial strains.

2.3. *Materials and methods*

All chemicals were commercially obtained in their purest form and used without purification. Solvents were redistilled by standard techniques before use [20]. The complexes were prepared by mixing cefepime · HCl (1 mmol) and metal salts: $MnCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, or $ZnCl_2$ (1 mmol) in MeOH (30 cm^3). The reaction mixture was then stirred at room temperature for ca 10 h, and a colored precipitate was formed. The precipitated complexes were filtered off, washed with water, methanol, and ether and dried under reduced pressure at room temperature. All syntheses were carried out under a nitrogen atmosphere. Polymeric complexes were formed, but on adding ethanol and scratching, the polymeric substances changed to a powder.

3. Results and discussion

Cefepime has a quaternary nitrogen that is positively charged at the *N*-methylpyrrolidine and also has two ionizable groups; one free carboxylate and NH_2^+ ($pK_a = 1.39$ and 3.26) [21]; it thus exists predominantly as a zwitterion at physiological pH. The elemental analyses (table 1) agree well with a 1 : 1 metal-to-ligand stoichiometry for all the complexes. The general formula [M(cefepime)Cl_2] have been assigned to the complexes. The insolubility of the complexes suggests that they are polymeric compounds and air-stable solids. The manganese(II) and zinc(II) complexes are light cream, nickel(II) and copper(II) complexes are green and maroon in color, while the cobalt(II) complex is pink in color. The complexes are insoluble in water and other common organic solvents such as EtOH, benzene, acetone, acetonitrile, and ether, but are soluble in DMSO. The insolubility and high melting of the complexes (>300°C) suggest that they are polymeric [22]. The molar conductance values of the complexes measured at room temperature lie in the range 10–18 $\Omega^{-1}\,cm^2\,mol^{-1}$, indicating that all complexes are nonelectrolytes [23]. Thus, all the chlorides in the complexes are held in the coordination sphere of metal(II) ions. Thermograms of all the metal complexes

are similar and show that there are not endothermic decompositions attributable to water and solvent molecules. These thermal results are in accord with the proposed formula for the complexes and also reveal that they are stable. Attempts to form complexes of a well-defined stoichiometry, under the above-mentioned conditions, with iron(II), iron(III), mercury(II), lead(II), cadmium(II), and tin(II) ions were unsuccessful.

3.1. *IR spectra*

The IR spectra of cefepime and its complexes are similar and have been assigned mainly to the wavenumbers directly involved in complex formation. The main IR wavenumbers are recorded in table 2. Generally, the ring carbonyl absorption frequency will be shifted to higher wavenumbers as the ring becomes more strained. The lactam (C=O) band appears at 1770 cm^{-1} in the spectrum of cefepime, while the complexes show this band as shifted and overlapped with the amide carbonyl band. This amide C=O–NH band appears at 1670 cm^{-1} while the complexes show this band at ~1670–1680 cm^{-1}; suggesting that ligand coordination with these metal ions occurs through the oxygen from the lactam carbonyl group rather than the amide carbonyl group, where the shifting was not significant.

The band at 1600–1620 cm^{-1}, corresponding to the carboxylate asymmetrical stretching of the free ligands, is shifted (5–20 cm^{-1}) to lower wavenumbers in the spectra of the complexes indicating coordination through that group [24, 25]. A carboxylate ligand can bind to the metal either monodentate or bidentate, giving changes in the relative positions of the antisymmetric and symmetric stretching vibrations [26]. The IR spectra of the complexes give a separation value of >200 cm^{-1} suggesting monodentate carboxylate. The remaining carboxylate bands, ν_{sym}(COO), γ(COO), ω(COO), and ρ(COO), formerly at 1400, 785, 610, and 530 cm^{-1}, respectively, also change as a result of coordination.

The presence of ν(M–N) stretching vibrations in the 450–490 cm^{-1} range for the metal complexes (absent in the free ligand) provide evidence that the moiety is bonded to the metal ion through nitrogen. Coordination of the NH_2–thiazole to the metal is not the only explanation of these absorption bands, alternatively the N atom of CONH and C=N–OCH_3 could coordinate in solid complexes, however, steric constraints prevent coordination of these N atoms along with the COO and lactam C=O groups. Furthermore, the C–N–C stretching and the N–H stretching vibrations of the CONH residues observed in free cefepime at 1160 and 3230 cm^{-1}, respectively, either do not

Table 2. Main vibrational wavenumbers (cm^{-1}).

Compound	ν(C=O) lactam	ν(C=O) amide	ν(COO) assym	ν(COO) symm	$\Delta\nu$(COO)
Cefepime	1770	1670	1600	1390	210
[Mn(cefepime)Cl_2]		1670	1600	1390	210
[Co(cefepime)Cl_2]		1670	1600	1380	220
[Ni(cefepime)Cl_2]		1680	1590	1380	210
[Cu(cefepime)Cl_2]		1670	1580	1380	200
[Zn(cefepime)Cl_2]		1670	1600	1380	220

shift or show a slight shift in the metal complexes indicating that these N atoms are not involved in coordination.

These results suggest coordination by the ligand as a multidentate chelating agent. Bands in the 350–400 cm^{-1} region observed in the complexes, and absent in the free cefepime, are tentatively assigned to ν(M–O) vibrations.

3.2. *NMR spectra*

The ^{1}H-NMR spectrum of [Zn(cefepime)Cl_2] was recorded in DMSO-d_6. All other complexes are paramagnetic. The coordination of NH_2 to zinc is exhibited by a downfield shift ($\Delta\delta = 0.25$ ppm) compared to the free ligand (5.05 ppm). Aminothiazolyl ring proton shows a singlet at the most downfield position, 7.16 ppm. Two additional singlets appeared at 4.07 and 3.24 ppm assigned to O–CH_3 and N–CH_3, respectively.

Three groups of peaks given by N–CH–S and N–CH–CO on the β-lactamic ring and NH appeared at 5.82, 5.21, and 6.68 ppm, respectively. Two groups of four resonances consistent with an AB system attributed to S–CH_2 on the dihydrothiazine ring and CH_2–N were observed in the 4.20–4.74 and 3.60–3.90 ppm regions with coupling constants 12.0 and 8.5 Hz for J_{AB}. The presence of a multiplet in the 2.20–2.45 ppm region is assigned to N-methylpyrrolidine ring protons. In general, the ^{1}H-NMR spectrum of the zinc(II) complex slightly changed as compared with that of the corresponding ligand with signals shifting downfield, as expected, due to increased conjugation on coordination.

The ^{13}C-NMR spectra indicate coordination of cefepime to Zn(II) through the oxygen of COOH and lactam CO moieties. According to the ^{13}C-NMR data, chemical shifts at 169.6 and 166.4 ppm in the spectrum of cefepime are assigned to the carbons of COOH and lactam CO. In the spectrum of the complex these appear at 185.7 and 180.3 ppm, respectively, suggesting coordination through the oxygen of COOH and lactam CO.

3.3. *Electronic spectra*

The UV–Vis spectra of cefepime and its complexes in DMSO have absorption maxima at 255–265 nm assigned to a $\pi \rightarrow \pi^*$ transition in the N–C–S moiety [27, 28]. An intraligand band at 290–310 nm is related to the $\pi \rightarrow \pi^*$ transitions within the aminothiazolyl. The band at 320–330 nm is ascribed to an intraligand transition of the $n \rightarrow \pi^*$ type in accord with literature data for transitions due to sulfur [27, 29]. The result that bands due to sulfur are not shifted suggests that sulfur is not involved in coordination to metal.

The local symmetry around metal(II) is lower than perfect octahedral, therefore an accurate band assignment is not possible due to the multicomponent nature of the bands. The manganese(II) complex showed very weak absorption bands. The cobalt(II) complex has a low intensity absorption at 520 nm, presumably due to a d–d transition. The nickel(II) complex showed a broad absorption band at 410 nm and a weak band at 560 nm attributable to d–d transitions. Since the ligand does not show any bands above 330 nm, the broad band at 430 nm for the copper(II) complex is assigned to a ligand $\rightarrow$ copper(II) charge-transfer transition [30]. The copper(II) complex also

exhibits a d–d transition as a weak band at 610 nm suggesting a five- or six-coordinate copper(II) environment [30].

3.4. *Magnetic measurements*

From molar magnetic susceptibilities, corrected magnetic moments were calculated using Pascal's constants. 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 six- or five-coordinate geometries [31]. The manganese(II) complex has a magnetic moment of 5.72 BM, consistent with high spin d^5 systems with five unpaired electrons and an $S = 5/2$ ground state [32]. The experimental value obtained for the magnetic moment of cobalt(II) in [Co(cefepime)Cl_2] is 3.42 BM, while the calculated value for a d^7 high spin electronic distribution is 3.87 BM. The observed value is higher than the expected value for a square-planar geometry and lower than either tetrahedral or octahedral geometries [33, 34]. On the basis of other results we conclude that cobalt(II) in a five-coordinate geometry with a high spin configuration would be favored. The experimental value obtained for the magnetic moment for nickel(II) in [Ni(cefepime)Cl_2] complex is 3.10 BM, close to the expected value for five coordinate (3.20–3.40 BM) [33, 34]. For [Cu(cefepime)Cl_2] the experimental magnetic moment measured is 2.07 BM while the calculated one for a d^9 configuration is 1.73 BM, suggesting the presence of impurities in the complex. Although lowered moments can be accounted for by antiferromagnetic interactions between the ions, higher moments would require ferromagnetic interactions which are rarer [35].

The EPR spectrum at room temperature of the powder sample of the copper(II) complex only showed two peaks. The calculated g values, $g_{\parallel} = 2.26$ and $g_{\perp} = 2.07$, indicate that the unpaired electron most likely resides in the $d_x^2 - d_y^2$ orbital having $^2B_{1g}$ as a ground state term [36, 37]. EPR spectra of a powdered sample of the manganese(II) complex at room temperature was obtained, but there was simply a single broad band centered at 3100 G with $g = 2.015$. There was no evidence of fine structure due to ^{55}Mn (100% natural abundance, $I = 5/2$).

3.5. *Coordination sites*

The coordination chemistry of transition metal ions with ceftriaxone [14] and cefotaxime [18] antibiotics, which also contain the methoxyimino and the aminothiazolyl moieties, has been reported. In the present case, the cefepime dipolar ion, like ceftazidime [17], has a number of potential donor atoms that might be involved in coordination. Coordination of cefepime may occur through the carboxylate, lactam carbonyl, and aminothiazolyl moieties; thus, cefepime could be tridentate. The multidentate chelation would require some kind of polymeric structure in which the ligand bridged different metal centers, since these atoms would be incapable of chelating a single metal due to geometric constraints. It is feasible that the metal ions in the [M(cefepime)Cl_2] complexes (where M = Mn(II), Co(II), Ni(II), Cu(II), and Zn(II)) containing two coordinated chloride ions are five-coordinate. Figure 2 shows a suggested structure of [M(cefepime)Cl_2] complexes where the positive charges (a quaternary amine with one plus charge and the metal(II) cation) are balanced with

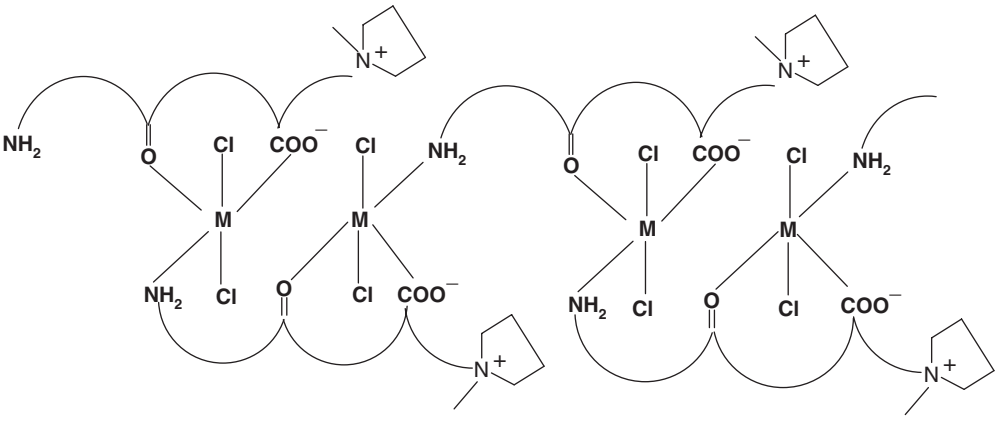

Figure 2. Suggested structure of [M(cefepime)Cl_2] complexes.

Table 3. Antibacterial activity of the cefepime metal complexes.

	Zone of inhibition (mm)				
Compound	E.C.	P.A.	S.T.	S.A.	B.C.
Cefepime	32	54	70	50	18
[Mn(cefepime)Cl_2]	0	0	0	0	0
[Co(cefepime)Cl_2]	0	34	35	28	18
[Ni(cefepime)Cl_2]	0	0	52	30	0
[Cu(cefepime)Cl_2]	0	0	32	30	0
[Zn(cefepime)Cl_2]	0	0	25	0	0

Notes: E.C. *Escherichia coli* ATCC 10536, P.A. *Pseudomonas aeruginosa* ATCC 9920, S.T. *Salmonella typhimurium* 10031, S.A. *Staphylococcus aureus* ATCC 6538, B.C. *Bacillus cereus* 9634. All doses were 400 µg per disc. Estimated error ±1 mm.

the negative charges (one carboxylate and two chloride anions). Despite the crystalline nature of the products, none proved suitable for X-ray structure determination.

3.6. *Microbiological screening*

The susceptibility of certain strains of bacteria toward cefepime and its metal complexes was judged by measuring the size of inhibition diameter. As assessed by color, the complexes remain intact during biological testing. The average results are shown in table 3.

Metal(II) complexes of cefepime were less active than free cefepime against all bacteria tested. The highest antibacterial activity was for the cobalt(II) complex against *P. aeruginosa*. In general, nickel(II), copper(II), and zinc(II) complexes had very poor activity against the tested microorganism and manganese(II) complex showed no activity at all. Though some trends in metal-based bactericidal agents are noted, it does not seem to be possible to correlate the bactericidal activity with metal complex structure in any simple way.

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