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Tridentate hydrazone metal complexes derived from cephalexin and 2–hydrazinopyridine: Synthesis, characterization and antibacterial activity

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Abstract: Metal(II) coordination compounds of a tridentate hydrazone ligand (HL) derived from the condensation of cephalexin antibiotic with 2–hydrazinopyridine were synthesized. The hydrazone ligand and mononuclear [ML(OAc)(H_2O)] (M(II) = Mn, Co, Ni, Cu, Zn, Ag) complexes were characterized by several techniques, including elemental and thermal analysis, molar conductance and magnetic susceptibility measurements, electronic, FT–IR, EPR and ^{1}H NMR spectral studies. The cephalexin 2–pyridinylhydrazone ligand HL behaves as a monoanionic tridentate NNO chelating agent. The biological applications of complexes have been studied on three bacteria strains (*Escherichia coli, Acinetobacter baumannii* and *Enterococcus faecalis*) by agar diffusion disc method.

Keywords: hydrazone metal complexes, cephalexin 2–pyridinylhydrazone ligand, synthesis hydrazone containing cephalexin, magnetic and spectral studies, antibacterial activity

Introduction

In view of their applicability in various fields, hydrazones, a member of the Schiff base family with triatomic >C=N–NH– linkage, takes the forefront

position in the development of coordination chemistry. Reports on the synthesis, characterization and structural studies on hydrazone ligands show the importance of hydrazone complexes in various fields, including analytical and biological fields [1, 2]. Many studies have been reported regarding the biological activities of hydrazones, including their anticancer [3–5], antibacterial [6, 7] and antidepressant activities [8].

The β–lactam ring, has largely been recognized as a useful building block in the synthesis of biologically important compounds. The activity of famous antibiotic classes such as the penicillins and cephalosporins is attributed to the presence of a β–lactam ring [9]. Unfortunately, the wide use of the antibiotics resulted in the serious medical problem of drugs resistance and public health concern [10, 11]. Preparation of new synthetic derivatives of antibiotics with novel mechanism of action has become an important task to cope with drug resistance problems.

Although hydrazones have extensively been studied and widely employed in coordination chemistry, rather less is known about hydrazone ligands containing β–lactam antibiotics. Continuing with metal–based antibiotics studies in order to establish whether complexation affects the pharmacological properties of the ligand and to derive additional fundamental knowledge about antibiotic action [12–16], we report here the isolation and characterization of metal(II) complexes containing a hydrazone ligand derived from the condensation of cephalexin antibiotic, first generation cephalosporin, with 2–hydrazinopyridine. The chemical structure of cephalexin is shown in Figure 1.

Experimental

Materials and methods

All necessary precautions were taken to exclude oxygen and moisture during the synthesis and handling of the compounds. Analytical grade

chemicals were used as received for all experiments. Fourier transform infrared (FTIR) spectra of the ligand and its metal complexes as KBr pellets were recorded in the spectral range 4,000–400 cm^{-1} with a Perkin-Elmer Series 2000 apparatus. FT−IR spectra as polyethylene pellets were recorded between 450 and 120 cm^{-1} using a Bruker IFS 66V spectrophotometer. EPR spectra were recorded on a Bruker ECS 106 spectrometer operating in the X−band (9.76 GHz). DPPH free radical was used as the g marker. Measurements of d–d transitions in the visible and and u.v. regions were taken with a Perkin−Elmer spectrophotometer. The contents of C, H, N and S were analysed on a LECO CHNS 932 model microanalytical instrument. To determine the metal contents, the compounds were decomposed by wet digestion at 350 °C with sulphuric acid and hydrogen peroxide and determined by normal complexometric titration procedures with standard EDTA solution [17]. The metal contents as well as the coordinated water were also obtained from the TGA curves. Molecular weight determinations were carried out by the Rast camphor method. Magnetic susceptibilities were measured on a Johnson Matthey Magnetic Susceptibility balance at room temperature using $HgCo(NCS)_4$ as calibrant. ^1H NMR spectra were run at 80 MHz on a Varian spectrometer in DMSO against tetramethylsilane (TMS) as internal reference. Thermograms were recorded on a simultaneous thermal analyzer, STA−6000 (Perkin Elmer) instrument at a heating rate of 10 ºC min^{-1} up to 800 °C. X−ray powder diffraction patterns for the studied complexes and final solid product of thermal decomposition were recorded on a HZG 4 diffractometer. Measurements were taken over the range of 2θ = 2–70° using Ni filtered CuKα radiation.

Synthesis of 2−hydrazinopyridine

One mmol liquid 2−chloropyridine was dissolved in 2.0 mL 85% hydrazine hydrate and methanol (5 mL) and the mixture was refluxed at 90°C for 6 h upon cooling, a yellow solid was separated out and recrystallized with ethanol−DMF to get the pure 2−hydrazinopyridine. Yield 60%.

Synthesis of cephalexin 2−pyridinyl hydrazone HL

To 1 mmol of cephalexin in 25 mL of hot methanol were added 1 mmol of 2−hydrazinopyridine. The solution was refluxed under nitrogen atmosphere at 70 °C for 3 h to give a dark yellow precipitate. This material was filtered off and washed with methanol and ether, and dried under reduced pressure. The product was purified by recrystallization from the same solvent. Yield 54%.

Synthesis of hydrazone metal complexes

Metal(II) complexes were prepared by the same general method. To a solution of 1 mmol of the metallic acetate in 20 mL of water was slowly added with stirring a solution of 1 mmol of HL in 10 mL of ethanol. To this solution KOH (0.1% in methanol) was added to adjust the pH of the solution at 7–8 and the mixture was then refluxed for 4 h and coloured precipitates formed. The metal(II) complexes of HL were separated from the reaction mixture as amorphous solids and washed several times with water, methanol and ether and dried under reduced pressure at room temperature. Complexes were purified by recrystallization from dimethylsulfoxide/water mixture. Yield 65–78%,

Antibacterial activity

The antibacterial activity of hydrazone ligand and hydrazone metal complexes was tested against *Enterococcus faecalis* as a Gram−positive bacterium and *Escherichia coli* and *Acinetobacter baumannii* as

Gram–negative bacteria, according to a modified Kirby–Bauer disc diffusion method under standard conditions using Mueller–Hinton agar medium, as previously reported [12–15]. The test compounds in DMSO solutions (5 x 10^{-2} M) were added dropwise (10 μL) to a 5 mm diameter filter paper disc placed at the centre of each agar plate.

Results and discussion

The ligand and the metal(II) complexes were isolated pure in very good yields and they are of various colours. The ligand, manganese(II) and zinc(II) complexes are yellow, cobalt(II) complex is red wine, nickel(II) and copper(II) complexes are green, and silver(II) is orange in colour. All the complexes did not melt/decompose when heated up to 158 °C. The synthetic route of HL ligand is given in Scheme S1. The elemental analyses of the ligand and complexes are contained in Table 1 and they agree well with a 1:1:1:1 metal: ligand: acetate: coordinated water stoichiometry. Thus, the general formulae [ML(OAc)(H_2O)] (M(II) = Mn, Co, Ni, Cu, Zn, Ag) have been assigned to the complexes and they are very air stable solids at room temperature without decomposition for a long time. The complexes are insoluble in water and other common organic solvents such as ethanol, chloroform, benzene, acetone, dichloromethane, DMF, acetonitrile and ether but soluble in DMSO. Attempts to form complexes of a well–defined stoichiometry, under the above–mentioned conditions, with chromium(III), iron(II) and mercury(II) ions were unsuccessful. The molar conductance values measured in DMSO at room temperature vary from 10.4 to 12.8 S cm^2 mol^{-1}, revealing the non–electrolytic nature of the complexes [18].

Thermal analysis

By thermal analysis, information on their properties, nature of intermediate and final products of their thermal decomposition can be obtained [19]. From TGA curves, the mass loss was calculated for the different steps and compared with those theoretically calculated for the suggested formulae based on the results of elemental analyses as well as molar conductance measurements. TGA indicated the formation of metal oxide as the end product from which the metal content could be calculated and compared with that obtained from analytical determination. Thermograms of the hydrated metal complexes indicate endothermic decompositions in two steps and also reveal that the complexes are stable with no hydration water and solvent molecules. The first step in the 158 to 175 °C range is assigned to loss of coordinated water molecules (Table 1). The final decomposition step includes complete evaporation of the ligand as well as formation of metal oxide as final product from which the metal content was found to be in very good agreement with the data obtained from complexometric analyses. Thus, the overall thermogravimetric results are consistent with the formulation of these complexes. The solid residues obtained during thermal decomposition of complexes are suitable metal oxides: MnO, CoO, NiO, CuO and ZnO. Their compositions have been confirmed by X−ray diffraction measurement. The diffraction patterns of obtained residues have been compared with reference patterns.

^{1}H–NMR

The values of the chemical shifts obtained were similar to those of Schiff base ligands reported in the literature [20]. In the ^{1}H–NMR spectrum of hydrazone ligand single peaks attributed to methyl, COOH and =N–NH groups appeared at 1.95, 10.1 and 11.2 ppm respectively. Three groups of double peaks given by N=C–CH and N–CH on the β–lactam ring and O=C–NH– appeared at 4.90, 5.45 and 9.01 ppm, respectively. One group

of four resonance signals consistent with an AB system attributed to S–CH_2 on the dihydrothiazine ring, was observed in the 3.18–3.49 ppm region with coupling constant 16.9 Hz for J_{AB}. Furthermore, coupling between NH_2 and the adjacent CH moieties could not be distinguished and a broad single signal due to NH_2 protons was observed at 5.05 ppm. A multiplet in the range 6.70–7.90 ppm due to aromatic ring protons was also present. All the complexes are paramagnetic with the exception of zinc(II) complex, therefore the ^{1}H–NMR spectra of the complexes could not be obtained Comparison of the ^{1}H–NMR spectrum of hydrazone ligand with that of the diamagnetic zinc(II) complex, shows the absence of the proton signals assigned to the COOH moiety of hydrazone ligand indicating deprotonation and suggests the formation of COO–metal bond. No significant changes in ^{1}H chemical shifts were observed for other atoms upon complexation suggesting that, in solution, the aromatic rings are not involved in stacking interactions. Owing to their low solubility it was not possible to record satisfactory ^{13}C–NMR spectrum for the diamagnetic complex.

Infrared spectra

The IR spectrum of the complexes were recorded down to the far–IR region of 200 cm^{-1} and tentative band assignments of some characteristic bands of hydrazone ligand were made by analogy with other related systems [21, 22]. The main infrared spectral bands of the hydrazone ligand and its metal complexes are presented in Table 2. The lactam ν(C=O) band appear at 1735 cm^{-1} in the spectra of cephalexin. The IR spectrum of the hydrazone ligand shows no absorption bands which can be assigned to lactam ν(C=O) vibrational mode coming from cephalexin. The absence of such absorption together with the appearance of a new band at 1635 cm^{-1} attributed to ν(C=N–NH) vibrations, is consistent with the product being

the expected hydrazone ligand [23, 24]. The infrared spectra of the metal complexes display IR absorption bands in the 1620–1625 cm^{-1} range which can be assigned to the C=N stretching frequencies of the coordinated ligand (HL), strongly suggesting involvement of this group in coordination. The ν(N–N) of the hydrazone complexes are found at 1110 cm^{-1}. The increase in the frequency of this band in the spectra of the complexes, due to the increase in the bond strength, again confirms the coordination via the azomethine nitrogen [25].

Disappearance of the stretching frequency at 1690 cm^{-1} assigned to ν(COOH) in the ligand and appearance in the complexes of new ν_{asym} and ν_{sym} modes of the (COO^{-}) group indicates that the hydrazone has reacted. Since the carboxylate group can coordinate to the metal ion in either bidentate or monodentate fashion, the ''Δν criterion'' [$\Delta\nu = \nu_{asym}(COO) - \nu_{sym}(COO)$] was employed to determine the coordination mode of the carboxylate group. The hydrazone metal complexes exhibit strong bands corresponding to ν_{asym}(COO) at 1600–1605 and 1580–1585 cm^{-1}, and ν_{sym}(COO) at 1420–1425 and 1370–1375 cm^{-1}. The Δν value of 175–180 and 205–215 cm^{-1} in our complexes are very similar to that reported for the metal complexes with both bidentate μ_2–O,O' and monodentate carboxylate groups respectively [26], thus indicating the carboxylate groups behave both as bidentate [27, 28] and monodentate ligands.

The remaining carboxylate bands, namely γ(COO), ω(COO) and ρ(COO), formerly at 785, 610 and 530 cm^{-1}, respectively, also change as a result of coordination. Furthermore, the appearance of new bands in the 450–490 cm^{-1} ranges attributed to ν(M–N) stretching vibrations, observed in the spectra of the complexes (absent in the free ligand) provide evidence that the pyridine moiety can be bonded to the metal ion through the nitrogen atom. The metal(II) complexes also display bands in the 1590–1595 and

1015–1025 cm^{-1} ranges due to ν(C=N) of coordinated pyridine ring and pyridine ring breathing mode of vibration [29].

The bands in the 350–400 cm^{-1} region observed in the complexes, and absent in the free hydrazone ligand, are tentatively assigned to ν(M–O) vibrations. Medium intensity band appearing in the 2830–2950 cm^{-1} region corresponds to aliphatic ν(C–H), while aromatic ν(C–H) stretches appear in the 3000–3100 cm^{-1} region. A broad band centered at 3426 cm^{-1} for the complex can be assigned to the ν(OH) stretch of coordinated water molecules. These overall data suggest that the bonding sites are the pyridine nitrogen, azomethine nitrogen and carboxylate oxygen atoms are involved in coordination in the complexes and that the hydrazone behaves as a tridentate monoanionic NNO chelating agent.

Magnetic properties

Magnetic susceptibility is the degree of the magnetization of a material in response to a magnet. The method measures the Boltzmann occupation of all energy levels. From the molar magnetic susceptibility values, corrected magnetic moments were calculated using Pascal's constants [30]. The magnitudes of the magnetic moments for the paramagnetic complexes fall within the ranges associated with spin−free high spin ions in octahedral fields.The manganese(II) complex has a magnetic moment value of 5.90 μ_B which is typical of high spin d^5 systems with five unpaired electrons and S = 5/2 ground state. The cobalt(II) complex has a magnetic moment of 4.60 μ_B which is a typical value of a d^7 system with three unpaired electrons indicating a quartet state in an octahedral arrangement around the metal, as compared with the reported values for octahedral complexes of cobalt(II) (4.7–5.2 μ_B) [31]. The nickel(II) complex has a magnetic moment of 3.24 μ_B characteristic of two unpaired electrons and greater than the spin−only

value, presumably due to the orbital contribution resulting from the transfer of an electron from the $d_{x^2-y^2}$ orbital to the d_{xy} orbital. The complex therefore probably has distorted octahedral geometry. At room temperature a magnetic moment of 1.9–2.2 μ_B is usually observed for mononuclear copper(II) complexes, regardless of stereochemistry [31]. A magnetic moment of 2.10 μ_B is observed for the copper(II) compound in the solid state.

When silver(I) acetate or nitrate was added to an aqueous suspension or to a 50% MeOH solution of the hydrazone ligand, performed under aerobic conditions, we observed an obvious color change from light yellow to dark orange. This observation suggests the occurrence of oxidation process, (Ag(I) $d^{10} \rightarrow$ Ag(II) d^9 complex), induced by the oxygen in air at an observable and rather rapid rate, depending on conditions of concentration and temperature. The starting Ag(I)–material does not yield an EPR spectrum, however, its oxidation was followed by EPR spectroscopy which showed the gradual gain of the signal assigned to the oxidised product; on completion of the oxidation a EPR signal could be appreciated. The silver(II) complex, isolated as [AgL(OAc)(H_2O)], is an orange solid which can be conveniently recrystallized from water, alcohol or acetonitrile. The compound is paramagnetic, having an effective magnetic moment of 2.10 μ_B characteristic of one unpaired electron. Thus the magnetic data supports a mononuclear structure. In a control experiment it was shown that no EPR signal occurred when hydrazone ligand was employed in the absence of silver(I) ions. The propensity of divalent silver to act as an oxidazing agent is well documented [32] and silver(II) complexes have been rare and stable only when formed with ligands which could survive the strong oxidizing capabilities of silver(II). For the most part these ligands have been nitrogen containing unsaturated heterocycles, such as pyridines and related

compounds. Ligand oxidation did not occur in the above reaction mixtures as shown by IR and UV−Vis spectra, and the compound appears to be stable in the solid state. The formation of this novel complex illustrates further the effect of the exceptionally strong ligand field exerted by the hydrazone ligand. This serves to raise antibonding level $(d_{x^2-y^2})^*$ of the Ag(I) ion of hydrazone complex to such a high energy that electron is easily removed.

The powder EPR spectrum of the orange product at room temperature shows an axial spectrum with principal g−factor values at 2.17 and 2.04 for parallel and perpendicular tensors respectively, and they coincide with other reported values for silver(II) complexes [33−35]. The experimental hyperfine structure observed is presented in Figure 2. The spectrum is a superposition of the hyperfine doublet structure characteristic of the silver nuclear spin (I = 1/2) and the superhyperfine couplings due to ^{14}N nuclei (I = 1). The number of the superhyperfine lines due to azomethine and pyridine nitrogen nuclei coordinated to the silver center is expected to be two sets of five (according to the known rule for the number of the superhyperfine lines to be equal to 2nI + 1, where I is the nuclear magnetic moment and n is the number of the corresponding nuclei) with relative intensity of the superhyperfine lines, 1:2:3:2:1. It could be even more complicated considering the presence of superhyperfine structure due to magnetic interaction in both, parallel and in perpendicular directions, with the nuclei of silver, ^{107}Ag, I = 1/2, 51.82% and ^{109}Ag, I = 1/2, 48.18%, which have the magnetic dipole moment of –0.1135 and –0.1305, respectively. The EPR line has a width of ~ 550 G. The line width does not change very much with decreasing temperature (down to 120 K) and should be the result of the dipolar and exchange interactions between the silver ions. Superhyperfine structure is also observed at both temperatures.

From the g values of a d^9 metal complex, we can obtain very important information about the structure of the complex. The geometric parameter G, which is a measure of the exchange interaction between the silver(II) centers in the polycrystalline compound, is calculated using the equation: $G = (g_{\parallel} - 2.0023) / (g_{\perp} - 2.0023)$ for axial spectrum. If $G < 4.0$ considerable exchange interaction is indicated in the solid complex [36–39]. The G parameter has the value of 4.25 suggesting that there is no exchange interaction between silver(II) centres.

Electronic spectra

A long–term UV–Vis study was carried out to verify the stability of new complexes in DMSO solution. Compared with hydrazone ligand, it is significant to note that the absorption wavelengths of new complexes hardly varied for up to 1 month, meaning that new complexes were stable in DMSO solution. The electronic spectrum of the hydrazone ligand in DMSO solution showed three broad bands at 32,250, 30,300 and 27,780 cm^{-1}. The former two bands are due to the π–π* transitions within the aromatic rings and remains almost unchanged in the spectra of metal complexes, while the third band, due to the π–π* transition within the >C=N–NH– chromophore, is shifted to a longer wavelength as a consequence of coordination when binding with the metal atom, confirming the formation of metal complexes and reflecting that azomethine nitrogen is involved in coordination [40].

The UV–Vis spectra of the metal complexes in DMSO solutions present absorption maxima attributable to the hydrazone ligand together with the absorptions, around 25,000–12,500 cm^{-1}, due to ligand to metal charge transfer and d–d transitions of the metal in the complexes [41]. The manganese(II) complex shows a very broad absorption at 22,220–20,830

cm^{-1} probably due to the coincidence of charge transfer, d → π*, L → M and intraligand n → π* transitions [42, 43]. The visible region spectrum of the cobalt(II) complex indicates additional two bands at 25,640 and 20,000 cm^{-1}, attributed to metal–ligand charge and $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ (ν_2) transitions respectively, suggesting octahedral stereochemistry around the metal ion [44]. The UV–Vis spectrum of the nickel(II) complex presents two major absorptions maxima, at 25,000 and 18,182 cm^{-1} due to d–d bands which may be assigned, considering that the immediate coordination sphere of the metal is O_h symmetry, to the spin allowed transitions $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ (ν_3) and $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ (ν_2) respectively [45]. Ligand field parameters (10Dq, B, β,) and ν_1 have been calculated for Ni(II) complex by using ν_2 , ν_3 and the procedure of Lever [41]. The results are satisfactory owing to the ratio ν_2/ν_1 is found to be 1.57 as required for the octahedral Ni(II) complexes [46]. The values of ν_1 = 11,570 cm^{-1}, the magnetic moment ($\mu_{spin\ only} < \mu_{obs} \sim \mu_{S+L}$) and the calculated ligand field parameters of Dq = 1,157 cm^{-1}, B = 565 cm^{-1} and β = 0.54 are also consistent with the octahedral geometry. The low value of Racah parameter (B) for the complexes compared to the free ion value indicates significant covalent character of the metal–ligand bonds. The nephelauxetic ratio β also support covalent character in this complex. The electronic spectrum of copper(II) complex exhibits a broad band centered at 14,280 cm^{-1} (ε = 280.1 M^{-1} cm^{-1}) assigned to the $^2E_{2g} \rightarrow {}^2T_{2g}$ transition in a distorted octahedral geometry around the copper(II) ion. A band at 24,400 cm^{-1} is assigned to charge–transfer, mainly of the L → Cu type [41]. The electronic spectrum of silver(II) complex presents a broad band at 25,000 cm^{-1} assigned to charge transfer of the L → Ag type. The spectrum of the Zn(II) complex exhibits a strong intense charge transfer transition (LMCT) band at 27,000 cm^{-1}.

Coordination sites

The coordination chemistry of transition metal ions with ceftriaxone [47], cefotaxime [48], cefepime [49] and ceftazidime [50] antibiotics have been reported. In the present case, the hydrazone ligand containing cephalexin has a number of potential donor atoms in various positions which can bind to the metal ions forming multinuclear chelates. From the data it appears that each metal ion lies in a distorted octahedron coordination sphere and the hydrazone would act as an efficient pseudo–encapsulating ligand, with pyridine nitrogen, azomethine nitrogen and carboxylate oxygen atoms, presumably bound to the octahedral ions. Thus, the metal ions in the [ML(OAc)(H_2O)] complexes containing one water molecule and acetate with bidentate mode at the vertices of an octahedron are hexacoordinate. We have attempted to grow single crystals of the metal chelates but in no case have we had any success, due to their insolubility in common organic solvents. The complexes only form amorphous materials as revealed by their XRD patterns. Up to now no crystal structures of hydrazone complexes containing cephalexin have been reported. These studies represent a contribution to future crystallographic analyses, which are complicated by the difficulties in obtaining X–ray quality crystals of cephalosporin derived complexes. Although crystal structure of the complexes are not known, the coordination environment of mononuclear complexes, may tentatively be proposed (Figure 3).

Antibacterial activity

Preliminary screening for antimicrobial activities of the stock solutions were performed qualitatively using the disc diffusion assay. In vitro antimicrobial activities were measured from the diameter of clear inhibition zones caused by samples against the same bacteria and under the identical

experimental conditions. As assessed by colour, the complexes remain intact during biological testing. In order to clarify role of DMSO any participating and metal(II) acetate salts in the biological screening, separate studies were carried out with the solutions alone of DMSO and the free metal salt and they have been found that they have no effect on the growth of any microorganisms taken. The antibacterial activity of hydrazone ligand HL as well as its metal(II) complexes were tested on against *Enterococcus faecalis, Escherichia col*i and *Acinetobacter baumannii* bacteria and compared to cephalexin used as standard.

The average results are shown in Table 3 where can be appreciated that the hydrazone ligand and its metal complexes have different behaviour compared with standard antibiotics against the same bacteria. Thus: (1) Cephalexin and the hydrazone ligand have similar antibacterial activity. (2) Manganese(II), cobalt(II) and silver(II) complexes were found to have higher activity than the established drug and HL against the bacteria strains studied under the test conditions, showing that they have good activity as bactericides. (3) The antibacterial activity of nickel(II), copper(II) and zinc(II) complexes show to be less toxic than the reference drug and hydrazone ligand.

According to Tweedy's theory [51], the enlarged activity of complexes compared to ligands may be attributed to increased delocalisation of π electrons of ligand over the whole chelate ring. Chelation reduces the polarity of metal ions and increases the lipophilic character of the chelate, which subsequently favors its permeation through the lipid layers of the cell membrane and blocking the metal binding sites on enzymes of microorganism. However, in the present case, the in vitro antibacterial activities demonstrated that manganese(II), cobalt(II) and silver(II) complexes have higher antimicrobial activity in comparison with that of the ligand HL, whereas nickel(II), copper(II) and zinc(II) complexes showed

lower activity against the tested strains compared to hydrazone ligand. Therefore, antimicrobial activity must be influenced by other factors beyond membrane permeability.

The targets for β–lactam antibiotics are cell wall–synthesizing enzymes (penicillin binding proteins, PBPs) which are found as both membrane–bound and cytoplasmic enzymes that catalyze cross–linking reactions. β–lactam antibiotics, interfere with cell wall synthesis by binding covalently to the PBPs catalytic site. PBPs are present in almost all bacteria, but they vary from species to species differing in amount, molecular weight, affinity for β–lactam antibiotics and enzymatic function (e.g., transpeptidase, carboxypeptidase, or endopeptidase) [52].

The results in Table 3 can be understood considering that the enzyme probably serves primarily to hold catalytic groups or the substrate in the proper positions and is possible to expect that hydrazone metal complexes may change the stereochemistry required in solvolytic reactions on an enzyme surface. The obtained results may highlight that the bactericidal activity of hydrazone metal complexes compared to hydrazone ligand and cephalexin may reflect a different mechanistic pathway by which they react with the PBP active sites to achieve formation of a stable PBP–inhibitor adduct. The level of resistance to β–lactam metal complexes is determined by the amount, nature and kinetic properties of the PBPs.

Conclusions

A hydrazone ligand derived from cephalexin and 2–hydrazinopyridine and its transition metal complexes have been prepared. The hydrazone coordination to metal occurs through the pyridine–N, azomethine–N and carboxylate–O atoms. Manganese(II), cobalt(II) and silver(II) complexes were found to have higher bactericidal activity than the uncomplexed

cephalexin and the hydrazone ligand against the bacteria strains, showing that they have a good activity as bactericides. The nickel(II), copper(II) and zinc(II) complexes showed to be less toxic than the reference drug and the hydrazone ligand. Apart of membrane permeability, antibacterial activity of metal complexes depends mainly on the metal ion and the type of microorganism.

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Table 1. Analytical, thermoanalytical (TG) and physical data

Compound	Found (Calcd.) %							
	C	N	H	S	H_2O	M^1	M^2	Mol wt.
[(HL)]	57.6	19.2	4.4	7.2				427.0
[($C_{21}H_{21}N_6O_3S$)]	(57.7)	(19.2)	(4.8)	(7.3)				(437.5)
[MnL(OAc)(H_2O)]	48.7	14.9	4.9	5.4	2.8	9.5	9.7	553.2
[Mn($C_{23}H_{25}N_6O_6S$)]	(48.6)	(14.8)	(4.4)	(5.6)	(3.2)	(9.7)	(9.7)	(568.5)
[CoL(OAc)(H_2O)]	48.5	14.9	4.8	5.3	3.5	10.2	10.5	560.3
[Co($C_{23}H_{25}N_6O_6S$)]	(48.3)	(14.7)	(4.4)	(5.6)	(3.1)	(10.3)	(10.3)	(572.5)
[NiL(Oac)(H_2O)]	48.7	14.8	4.2	5.5	2.9	10.4	10.1	561.4
[Ni($C_{23}H_{25}N_6O_6S$)]	(48.3)	(14.7)	(4.4)	(5.6)	(3.1)	(10.3)	(10.3)	(572.3)
[CuL(OAc)(H_2O)]	47.7	14.2	4.7	5.4	3.5	10.7	11.4	564.8
[Cu($C_{23}H_{25}N_6O_6S$)]	(47.9)	(14.6)	(4.4)	(5.6)	(3.1)	(11.0)	(11.0)	(577.1)
[ZnL(OAc)(H_2O)]	48.1	14.7	4.1	5.8	3.2	11.2	11.0	565.3
[Zn($C_{23}H_{25}N_6O_6S$)]	(47.7)	(14.5)	(4.4)	(5.5)	(3.1)	(11.3)	(11.3)	(578.9)
[AgL(OAc)(H_2O)	44.8	13.2	3.8	5.4	3.0	17.1	17.7	610.7
[Ag($C_{23}H_{25}N_6O_6S$)]	(44.5)	(13.5)	(4.1)	(5.2)	(2.9)	(17.4)	(17.4)	(621.4)

M^1 = complexometric analysis, M^2 = thermal analysis

Table 2. Main vibrational wavenumbers of the metal complexes (cm^{-1})

Compound	νC=O amide	νC=N imino	νCOO asym	νCOO sym	Δν	νCOO asym	νCOO sym	Δν
Cephalexin	1657							
HL	1656	1635						
[MnL(OAc)(H_2O)]	1656	1625	1600	1420	180	1580	1370	210
[CoL(OAc)(H_2O)]	1653	1620	1600	1420	180	1585	1375	210
[NiL(OAc)(H_2O)]	1653	1620	1605	1425	180	1585	1370	215
[CuL(OAc)(H_2O)]	1658	1625	1605	1425	180	1580	1370	210
[ZnL(OAc)(H_2O)]	1654	1620	1600	1425	175	1580	1375	205
[AgL(OAc)(H_2O)]	1656	1620	1600	1425	175	1585	1375	210

Table 3 Antibacterial activity

Compound	Zone of inhibition (mm) E.C.	A.B.	E.F.
Cephalexin	40.0 ± 1.0	50.0 ± 1.0	20.0 ± 1.5
[HL]	38.0 ± 1.0	50.0 ± 1.0	21.0 ± 1.0
[MnL(Oac)(H_2O)]	45.0 ± 1.0	52.0 ± 1.0	30.0 ± 1.0
[CoL(OAc)(H_2O)]	45.0 ± 1.0	55.0 ± 1.0	50.0 ± 1.0
[NiL(OAc)(H_2O)]	15.0 ± 1.0	35.0 ± 1.0	5.0 ± 1.0
[CuL(OAc)(H_2O)]	20.0 ± 1.5	40.0 ± 1.0	5.0 ± 1.0
[ZnL(OAc)(H_2O)]	5.0 ± 1.0	10.0 ± 1.0	5.0 ± 1.0
[AgL(OAc)(H_2O)]	42.0 ± 1.0	52.0 ± 1.0	25.0 ± 1.0

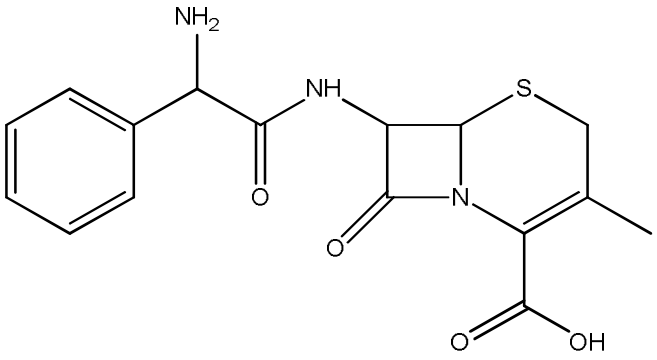
E.C. Escherichia coli, A.B. Acinetobacter baumannii, E.F. Enterococcus faecalis. Values are the mean ± standard deviation of the mean.

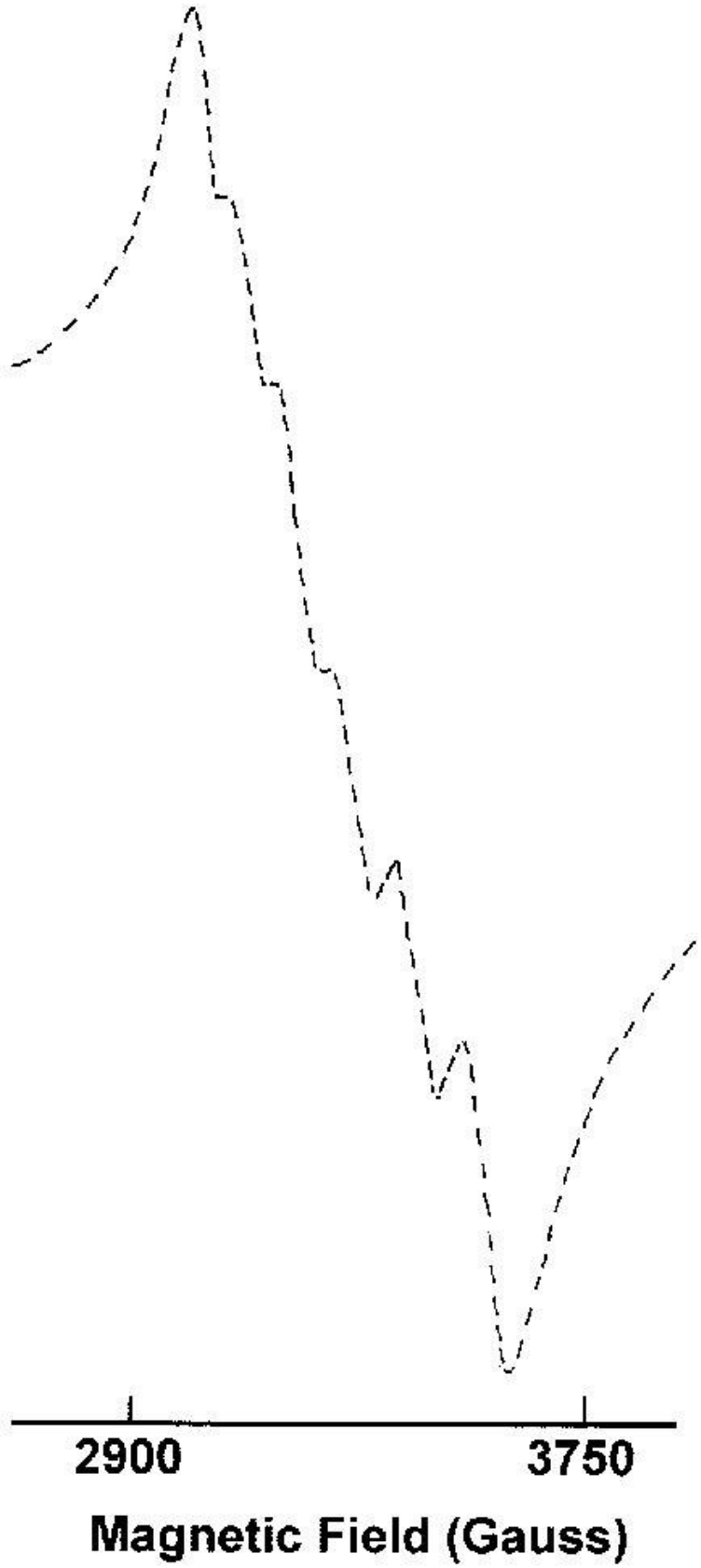
FIGURE CAPTIONS

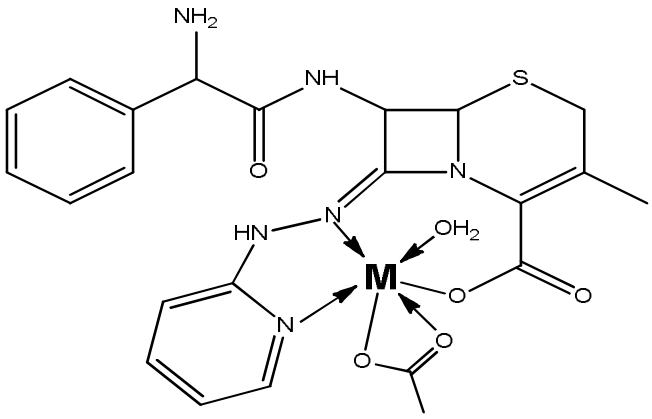
Figure 1. Chemical structure of cephalexin

Figure 2. EPR spectrum of [AgL(OAc)(H_2O)] complex at room temperature

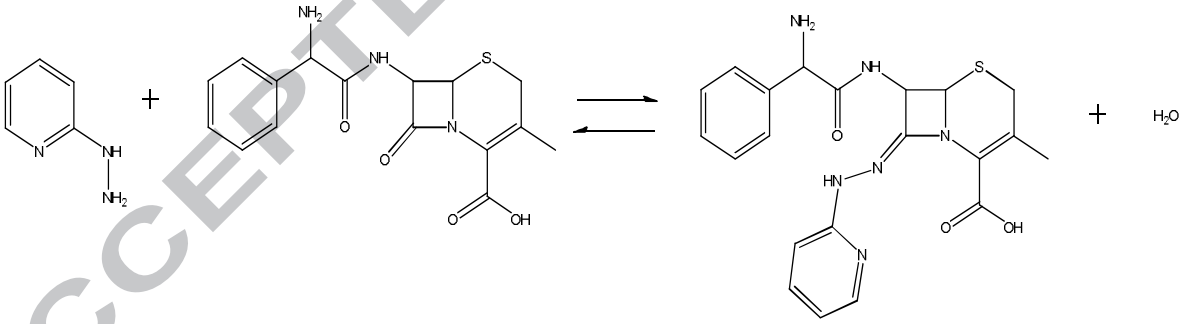
Figure 3. Proposed structure of [ML(OAc)(H_2O)] complexes







Supplementary data



Scheme S1

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Graphical abstract

Tridentate hydrazone metal complexes derived from cephalexin and 2–hydrazinopyridine: Synthesis, characterization and antibacterial activity

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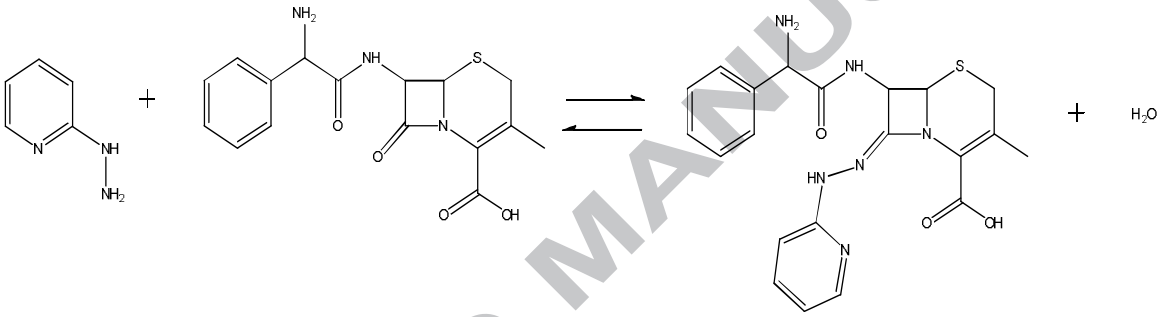
Transition coordination compounds with a tridentate hydrazone ligand (HL) derived from the condensation of cephalexin antibiotic with 2–hydrazinopyridine were synthesized, characterized and screened for antibacterial activity.



Spectrochimica Acta Part A

Highlights

A new Schiff base using cephalexin antibiotic was prepared

Cephalexin Schiff base transition metal complexes were prepared.

Magnetic, spectroscopic properties and antibacterial activity were studied