Inorganica Chimica Acta 421 (2014) 59–66

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Characterization and biological activity of Pefloxacin–imidazole mixed ligands complexes

Amina A. Soayed [a,b,*], Heba M. Refaat [a], Doaa A. Noor El-Din [c]

[a] Faculty of Science, Alexandria University, Chemistry Department, P.O. Box 426, Ibrahimia, Alexandria 21321, Egypt
[b] University of Dammam, Chemistry Department, Saudi Arabia
[c] Stability Laboratory, Medizen Pharmaceutical Industries, Alexandria, Egypt

ARTICLE INFO

Article history:
Received 11 February 2014
Received in revised form 28 April 2014
Accepted 12 May 2014
Available online 2 June 2014

Keywords:
Pefloxacin
Imidazole
IR
Thermal studies
Biological activity

ABSTRACT

Solid complexes $[M(PEF)(HIm)Cl_x(H_2O)_m]\_nH_2O$, [M = Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cr(III)] and $[Ag(PEF)(HIm)]\cdot 2H_2O$; PEF = Pefloxacin, HIm = imidazole were prepared and characterized using elemental analyses, IR spectroscopy, conductance measurements, UV–Vis spectra, ESR of the copper complex, magnetic moments measurements and ^1H NMR spectra. The results have shown that Pefloxacin reacts as a bidentate ligand and is bound to the metal ions through the pyridone oxygen and one carboxylic oxygen, except for the silver(I) complex where the metal ion is chelated to the piperazinyl nitrogen. Thermal studies including TGA and DTA were also performed on the complexes in order to study their stabilities and activation energies, ΔE^*, entropies ΔS^*; enthalpies ΔH^* and order of reactions n have been derived using Horowitz–Metzeger method calculations. The mixed ligands complexes were evaluated for their antibacterial activity against two bacterial species, namely *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*). Antifungal screening was studied against two species (*Aspergillus flavus* and *Candida albicans*). The complexes under investigation were found to possess better antibacterial effects than uncomplexed Pefloxacin as well as antifungal activity.

1. Introduction

It is well documented that antibiotics are capable of inhibiting the growth of and even destroying bacteria and other microorganisms. One class of these antibiotics are fluoro-quinolones, a relatively new group of antibiotics. It is the fastest growing antibacterial class, increasingly being used in both hospital and community sectors to treat a broad range of infections [1]. They constitute a family of synthetic, broad-spectrum antibacterial agents with bacterial activity [2]. The first fluoroquinolones were used for the treatment of serious infections caused by gram-negative organisms, including Pseudomonas species [3], urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexually transmitted diseases, prostatitis, community acquired pneumonia, acute bronchitis and sinusitis [3]. The newer fluoroquinolones have a wider clinical use and a broader spectrum of antibacterial activity including gram-positive and gram-negative aerobic and anaerobic organisms and in the treatment of community-acquired pneumonia and intra-abdominal infections [2]. Pefloxacin (PEF), [1-ethyl-6-fluoro-7-(4-methyl-piperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid], which is a synthetic chemotherapeutic agent used to treat severe and life-threatening bacterial infections and is a member of the broad spectrum fluoroquinolone antibacterial agents, has an excellent antibacterial activity against most gram-negative and gram-positive bacteria. Its action results from interference with the activity of DNA gyrase and topoisomerase IV, which are needed for the transcription and replication of bacterial DNA. Pefloxacin also demonstrates favorable cellular penetration characteristics, yielding high tissue/serum ratios [4]. Pefloxacin also shows a good absorption with high bioavailability, a long half-life, excellent tissue and body fluid penetration.

In an extension of our studies on mixed ligand complexes containing fluoro-quinolones [5], we hereby report the characterization and biological effect of Pefloxacin–imidazole mixed ligands complexes. The choice of imidazole was because of its being a polar and ionizable aromatic compound which improves pharmacokinetic characteristics and is thus used as a remedy [6,7]. The high therapeutic properties of the imidazole related drugs have encouraged medicinal chemists to synthesize a large number of novel chemotherapeutic agents remedying various dispositions in

* Corresponding author at: Faculty of Science, Alexandria University, Chemistry Department, P.O. Box 426, Ibrahimia, Alexandria 21321, Egypt.
E-mail addresses: amsoayed@yahoo.com, aminasoayed@yahoo.com (A.A. Soayed).

http://dx.doi.org/10.1016/j.ica.2014.05.020
0020-1693/

clinical medicines [8]. Complexes of imidazole with transition metal ions are also of interest because of their close relationship with biological systems involving histidine residues [9].

2. Experimental

2.1. Materials

Pefloxacin (PEF), was purchased from Sigma. Imidazole (HIm), $CoCl_2{\cdot}6H_2O$, $CuCl_2{\cdot}H_2O$, $CrCl_3{\cdot}6H_2O$,$NiCl_2{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$, $ZnCl_2$ anhydrous, $AgNO_3$ anhydrous and all solvents (DMSO and CH_3OH) were purchased from Aldrich Chemical Co. All the chemicals and solvents are analytical reagent grade and were used as purchased without further purification.

2.2. Synthesis of Pefloxacin–imidazole metal complexes

The dark green cobalt Pefloxacin–imidazole complex $[Co(PEF)(HIm)Cl(H_2O)_2]$ was prepared by dissolving $CoCl_2.6H_2O$ (1 mmol, 0.23793 g) in 50 ml of distilled water and the solution was added to a mixture of (1 mmol, 0.3335 g) of Pefloxacin mesylate dissolved in 10 ml distilled water together with (1 mmol, 0.04 g) of NaOH and (1 mmol, 0.068 g) of imidazole in 10 ml of distilled water. The reaction mixture was refluxed for 2 h. The solution was left for slow evaporation and after few days the product was deposited, collected with filtration, washed with distilled water several times and dried over calcium chloride in a desiccator.

The nil-blue, postage-green, dark-green, white, brownish-yellow, grey solid complexes of $[Cu(PEF)(HIm)Cl]2.5H_2O$, $[Ni(PEF)(HIm)Cl]{\cdot}H_2O$, $[Cr(PEF)(HIm)Cl_2(H_2O)]$, $[Zn(PEF)(HIm)Cl(H_2O)_2]{\cdot}0.25H_2O$, $[Mn(PEF)(HIm)Cl]$ and $[Ag(PEF)(HIm)]{\cdot}2H_2O$ were prepared in a similar manner described above by using methanol as a solvent and using $CuCl_2{\cdot}2H_2O$, $NiCl_2{\cdot}6H_2O$, $CrCl_3{\cdot}6H_2O$, $ZnCl_2$ anhydrous, $MnCl_2{\cdot}4H_2O$ and $AgNO_3$ anhydrous in 1:1:1 ratios, respectively. All complexes are soluble in DMSO and DMF and are non-electrolytes.

Anal. Calc. for $[Cr(PEF)(HIm)Cl_2(H_2O)]$: C, 44.280; H, 4.612; N, 12.915; Cl, 13.099; Cr, 9.594. Found: C, 44.201; H, 4.481; N, 13.211; Cl, 12.812; Cr, 9.662%. $[Mn(PEF)(HIm)Cl]$: C, 48.885; H, 4.680; N, 14.244; Cl, 7.223; Mn, 9.721. Found: C, 48.504; H, 4.901; N, 14.491; Cl, 7.445; Mn, 10.008%. $[Co(PEF)(HIm)Cl(H_2O)_2]$: C, 45.161; H, 5.080; N, 13.172; Cl, 6.680; Co, 11.089. Found: C, 44.933; H, 5.359; N, 13.328; Cl, 6.878; Co, 11.285%. $[Ni(PEF)(HIm)Cl]{\cdot}H_2O$:C, 46.765; H, 4.871; N, 13.640; Cl, 6.930; Ni, 11.438. Found: C, 46.501; H, 4.699; N, 13.821; Cl, 7.140; Ni, 11.181%. $[Cu(PEF)(HIm)Cl]{\cdot}2.5H_2O$:C, 44.033; H, 5.137; N, 12.843; Cl, 6.513; Cu, 11.657. Found: C, 44.360; H, 5.410; N, 12.588; Cl, 6.770; Cu, 11.871%. $[Zn(PEF)(HIm)Cl(H_2O)_2]{\cdot}0.25H_2O$: C, 44.248; H, 4.978; N, 12.905; Cl, 6.545; Zn, 12.056. Found: C, 44.511; H, 5.301; N, 13.283; Cl, 6.822; Zn, 12.148%. $[Ag(PEF))(HIm)]{\cdot}2H_2O$:C, 44.125; H, 4.964; N, 12.870; Ag, 19.838. Found: C, 44.311; H, 4.688; N, 13.012; Ag, 19.636%.

2.3. Instrumentation-physical measurements

Elemental C, H, N and halogen analyses were carried out on a Perkin Elmer CHN 2400. The percentage of the metal ions were determined by EDTA titration method as well as atomic absorption method using a Perkin Elmer A Analyst 400 model. UV–Vis spectra were done using a PYE-UNICAM SP 1900 Spectrophotometer at a concentration range of 5×10^{-4}–10^{-3}M. The aqueous sample is drawn into the assembly by passing a high-pressure stream of compressed air past the end of a capillary tube immersed in the sample. When the sample exits the nebulizer it strikes a glass impact bead, converting it into a fine aerosol mist within the spray chamber. The aerosol mist is swept through the spray chamber by the combustion gases—compressed air and acetylene in this case—to the burner head where the flame's thermal energy desolvates the aerosol mist to a dry aerosol of small, solid particles. The flame's thermal energy then volatilizes the particles, producing a vapor consisting of molecular species, ionic species, and free atoms.

Infrared (IR) spectra were recorded on a Perkin Elmer –FTIR 100 Spectrophotometer with samples prepared as KBr pellets in the range from 4000–400 cm^{-1}. TGA, DTG and DTA measurements were carried out under N_2 atmosphere (flow rate 20 ml/min), using 6 mg samples packed in an aluminum crucible. Samples were heated at 10 °C/min within the temperature range from room temperature to 800 °C using 60H Shimadzu detector.

Magnetic measurements were carried out on a Sherwood scientific magnetic balance using Gouy's method where Hg[Co(SCN)] was used as a calibrant. Electric conductance measurements were carried out with a GLP31-CRISON model and a C cell type, which had a cell constant of 12.88. Molar conductivities of solution of the ligand and metal complexes were measured in DMSO at 10^{-3} M. X-band electron paramagnetic resonance (EPR) spectra of the Cu(II) complex was carried out at liquid helium temperatures and recorded on a Bruker ER 200D EPR spectrometer equipped with an Oxford Instruments ESR900 cryostat and an Anritsu microwave frequency counter. All measurements were carried out at ambient temperatures with freshly prepared solution.

2.4. Antimicrobial investigation

Antimicrobial activities of PEF and the mixed ligands metal complexes were conducted using a modified Kirby-Bauer disc diffusion method [10] against different bacterial species, such as *Staphylococcus aureus* (*G+*) *and Escherichia coli* (*G−*) and fungus species, such *as Aspergillus flavus* and *Candida albicans* and compared with the reference drug free fluoroquinolones. The Müller–Hington agar was prepared and then cooled to 47 °C and seeded with tested microorganisms. After solidification, 5 mm diameter holes were punched by a sterile cork-borer. The investigated compounds, i.e., ligands and their complexes, were introduced in holes (only 100 μl) after being dissolved in DMSO at 10^{-4} M. These were grown in 10 ml of fresh media until they reached a count of approximately 108 cells/ml for bacteria or 105 cells/ml for fungi [11]. Plates were incubated with filamentous fungi as *Aspergillus flavus* at 25 °C for 48 h; Gram (+) bacteria as *Staphylococcus aureus* and (G−) *bacteria as Escherichia coli* they were incubated at 35–37 °C for 24–48 h and yeast as *Candida albicans* incubated at 30 °C for 24–48 h. The activity was determined by measuring the diameter of the inhibition zones (in mm) [10]. Growth inhibition was calculated with reference to the positive control, i.e., Pefloxacin (PEF).

3. Results and discussion

3.1. Microanalyses and molar conductivity studies

Microanalyses suggested the formation of $[M(PEF)(HIm)Cl_x(H_2O)_m]{\cdot}n(H_2O)$ complexes, where PEF = Pefloxacin, HIm = imidazole, M = Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Ag(I), (Table 1). The conductivity measurements of complexes showed them all to be non-electrolytes. According to literature, the measured values were found to be below the expected values for electrolytes [12].

3.2. Infrared Spectra of PEF–Him mixed ligands and their metal(II) complexes

The IR spectra of Pefloxacin mesylate (PEF) and imidazole (HIm) ligands were compared to those of the metal complexes to show