Synthesis and characterization of mixed ligand complexes of lomefloxacin drug and glycine with transition metals. Antibacterial, antifungal and cytotoxicity studies

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Mixed ligand complexes derived from lomefloxacin (LFX, L1) as primary ligand and glycine (L2) as secondary ligand have been prepared and characterized by conventional techniques including elemental analyses, XRD, infrared, electronic spectra, molar conductivity and thermal analyses. The elemental analyses data display the formation of 1:1:1 [M:L1:L2] complexes. The diffused reflectance and magnetic moment measurements reveal the presence of the complexes in an octahedral geometry. The infrared spectral data show that the chelation behavior of the ligands toward transition metal ions is through carbonyl O, and carboxylate O of LFX whereas the amino acid coordinate through the carboxylate oxygen and the amino nitrogen. The electronic spectral results display the existence of π–π* (phenyl rings), π–π* (NH2 and −C=O) and confirm the mentioned structure. The molar conductivity reveals an electrolytic nature of all chelates. The thermogravimetric analysis data of the complexes displays the existence of hydrated and coordinated water molecules. The effect of LFX, glycine and their complexes on the inhibition of bacteria or fungi growth were evaluated. The prepared complexes were found to exhibit enhanced activity on bacteria or fungi growth compared to LFX and glycine ligands. LFX, [Mn(LFX)(Gly)(H2O)2]Cl, [Co(LFX)(Gly)(H2O)2]Cl and [Zn(LFX)(Gly)(H2O)2]Cl were found to be very active against breast cancer cells with IC50 values 14, 11.2, 13 and 16.8, respectively, while glycine and other complexes had been shown to be inactive at lower concentration than 100 μg/ml.

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1. Introduction

Metal ion-mediated reactions involving nucleic acid constituents and amino acid side chains have been the subject of several investigations [1–6]. These reactions provide an opportunity to identify the nature of such interactions in vivo as they serve as models for many metalloenzyme reactions. The fluoroquinolones constitute an important class of synthentic antimicrobial agents, which have been the objects of intensive study [7]. Numerous studies regarding the interaction between various quinolones with metallic cations have been reported in the literature [8–11]. These studies have been primarily directed towards the identification of functional groups directly linked to the metal and to establish the structure formed by these coordination complexes. Other investigations aim to show the effect of metal ions upon antibacterial activity [12,13]. Still, other research groups have reported the antibacterial properties of metal complexes with fluoroquinolones [14–17]. The coordination of the fluoroquinolones with metallic ions by way of the piperazine nitrogen atoms is much less common. The literature contains few examples, such as a complex of zinc whose structure was revealed by X-ray diffraction, where two molecules of the ligand norfloxacin coordinate via the ketonic and carboxylic oxygen atoms and two other molecules coordinate by way of the terminal nitrogen of the piperazine [18,19]. Lomefloxacin is a bactericidal fluoroquinolone agent with activity against a wide range of gram-negative and gram-positive organisms. Its chemical IUPAC name is 1-ethyl-6,8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (Fig. 1). The bactericidal action of lomefloxacin results from interference with the activity of the bacterial enzymes DNA gyrase and topoisomerase IV, which are needed for the transcription and replication of bacterial DNA [1]. Modifications of LFX were made based on structure–activity relationships (SARs). It was discovered that a fluorescein atom at position 6 and a piperazine ring at position 7 greatly enhance the spectrum of activity. Recently, a relatively new approach to the rational design of antitumor agents has been introduced based on some new quinolone molecules that display a novel mode of action [2,3].

In order to gather more information, we thought it was important to investigate the interaction of lomefloxacin and amino acid (glycine) with the biologically important metal ions, Fe(III), Cr(III), Mn(II), Zn(II), Cu(II), Ni(II) and Co(II). These complexes were
prepared as models which might correspond to the complexes formed with fluoroquinolone drugs in vivo. The complexes were characterized based on elemental analysis, conductivity data, infrared spectra, electronic spectra and magnetic susceptibility data and their bonding modes assigned. LFX acts as a neutral bidentate ligand coordinating through the carboxylate oxygen and the amino nitrogen. Octahedral geometry for metal chelates is proposed.

2. Experimental

2.1. Materials

All chemicals and reagents used in this investigation were laboratory pure (BDH, Aldrich or Sigma) including CrCl$_3$6H$_2$O, FeCl$_3$6H$_2$O, CoCl$_2$6H$_2$O, NiCl$_2$6H$_2$O, CuCl$_2$2H$_2$O, ZnCl$_2$2H$_2$O, UO$_2$(NO$_3$)$_2$2H$_2$O, ThCl$_4$, glycine, NH$_4$OH, DMF, C$_2$H$_5$OH and double distilled water.

2.2. Solutions

A fresh stock solution of 1 $\times$ 10$^{-3}$ M LFX (0.351 g/l) was prepared in the appropriate volume of absolute ethanol. 1 $\times$ 10$^{-3}$ M Stock solutions of the metal salts (Fe(III), 0.27 g/l; Co(II), 0.23 g/l; Ni(II), 0.23 g/l; Cu(II), 0.17 g/l; Zn(II), 0.172 g/l; Cr(III), 0.26 g/l; UO$_2$(II), 0.50 g/l, Mn(II), 0.16 g/l and Th(IV), 0.37 g/l) were prepared by dissolving the accurately weighed amounts of the metal salts in the appropriate volume of de-ionized water. Acid mixture (0.04 M phosphoric, acetic and boric acids) from which series of universal buffer solution of different pH values (2–12) were prepared by using 0.1 M sodium hydroxide solution for adjusting the desired pH.

Dimethylsulphoxide (DMSO) (was supplied from Sigma Chemical Co., St. Louis, Mo, USA) was prepared in cryopreservation of cells. RPMI-1640 medium (Sigma Chemical Co., St. Louis, Mo, USA) was used. The medium was used for culturing and maintenance of the human tumor cell lines. The medium was supplied from Sigma Chemical Co., St. Louis, Mo, USA and were used for the supplemention of RPMI-1640 medium prior to use. 0.025% (w/v) Trypsin (Sigma Chemical Co., St. Louis, Mo, USA) was used for the harvesting of cells. 1% (v/v) Acetic acid (Sigma Chemical Co., St. Louis, Mo, USA) was used for dissolving the unbound SRB dye. 0.4% Sulphorhodamine-B (SRB) (Sigma Chemical Co., St. Louis, Mo, USA) dissolved in 1% acetic acid was used as a protein dye. A stock solution of trichloroacetic acid (TCA, 50%, Sigma Chemical Co., St. Louis, Mo, USA) was prepared and stored. Fifty microliters of the stock was added to 200 µl RPMI-1640 medium/well to yield a final concentration of 10% used for protein precipitation. 100% isopropanol and 70% ethanol were used. Tris base 10 mM (pH 10.5) was used for SRB dye solubilization. 121.1 g of tris base was dissolved in 1000 ml of distilled water and pH was adjusted by HCl acid (2 M).

2.3. Measurements

Microanalyses of carbon, hydrogen and nitrogen were carried out at the Microanalytical Center, Cairo University, Egypt, using CHNS-932 (LECO) Vario Elemental Analyzer. The X-ray powder diffraction analyses were carried out by using Philips Analytical X-ray BV, diffractometer type PW 1840. Radiation was provided by copper target (Cu anode 2000 W) high intensity X-ray tube operated at 4050 kV and 25 mA. Divergence and the receiving slits were 1 and 0.2, respectively. Analyses of the metals followed the dissolution of the solid complex in concentrated HNO$_3$, neutralizing the diluted aqueous solutions with ammonia and titrating the metal solutions with EDTA. Electronic and diffused reflectance spectra were recorded at room temperature on a Shimadzu 3101pc spectrophotometer as solutions in ethanol and solid complexes (BaSO$_4$ disk), respectively. $^1$H NMR spectra, as a solution in DMSO-d$_6$ were recorded on a 300 MHz Varian-Oxford Mercury at room temperature using TMS as an internal standard. The molar magnetic susceptibility was measured on powdered samples using the Faraday method. The diamagnetic corrections were made by Pascal’s constant and Hg[Co(SCN)$_4$] was used as a calibrant. pH measurements were carried out using Jenway 3505 pH meter. UK FT-IR spectra were recorded on a Perkin-Elmer 1650 spectrophotometer (4000–400 cm$^{-1}$) in KBr pellets. The antibacterial and antifungal activities were evaluated at the Microbiological laboratory, Microanalytical Center, Cairo University, Egypt. The antiancancer activity was performed at the National Cancer Institute, Cancer Biology Department, Pharmacology Department, and Cairo University, Egypt. The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (Meter tech, S 960, USA). Molar conductivities of $10^{-3}$ M solutions of the solid complexes in DMF were measured on the using Jenway 4010 conductivity meter. The thermal analyses (TG, DTG and DTA) of the solid complexes were carried out from room temperature to 800 °C using a Shimadzu TG-50H thermal analyzer.

2.4. Preparation of mixed ligand complexes

The present mixed complexes were prepared by mixing equal amounts (0.01 mol) of hot saturated ethanolic solution of the first ligand (lomefloxacin) and second ligand (glycine) with the same ratio of metal chloride or nitrate salts. The mixture was refluxed for 3 h. The resulting complexes were filtered and washed several times with hot ethanol until the filtrates become clear. The solid complexes then dried in desiccator over anhydrous calcium chloride. The yield ranged from 74% to 91%.

M(II) or M(III) or M(IV) + L$^1$ + L$^2$ $\rightarrow$ [ML$^1$L$^2$]$^+$

M(II) = Mn(II), Co(II), Ni(II), Cu(II), Zn(II), M(III) = Cr(III), Fe(III), M(IV) = Th(IV), L$^1$ = lomefloxacin, L$^2$ = glycine.
The dried complexes were subjected to elemental and spectroscopic analyses. The obtained complexes are soluble in ethanol, DMF and DMSO. All melting points of the complexes were measured and found to be >200 °C.

2.5. Biological activity

A filter paper disk (5 mm) was transferred into 250 ml flask containing 20 ml of working volume of tested solution (100 g/ml). All flasks were autoclaved for 20 min at 121 °C. LB agar media surfaces were inoculated with two investigated bacteria (gram positive and gram negative) and two strains of fungi then, transferred to a saturated disk with a tested solution in the center of Petri dish (agar plates). Finally, all these Petri dishes were incubated at 25 °C for 48 h where clear or inhibition zones were detected around each disk. Control flask of the experiment was designed to perform under the same condition described previously for each microorganism but with dimethylformamide solution only and by subtracting the diameter of inhibition zone resulting with dimethylformamide from that obtained in each case, so antibacterial activity could be calculated [20]. All experiments were performed as triplicate and data plotted were the mean value.

2.6. Anticancer activity

Potential cytotoxicity of the compounds was tested using the method of Skehan et al. [21]. Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compounds under investigation (0, 5, 12.5, 25, 50 and 100 μg/ml) were added to the cell monolayer triplicate wells were prepared for each individual dose. The monolayer cells were incubated with the compounds for 48 h at 37 °C and in 5% CO2 atmosphere. After 48 h, cells were fixed, washed and stained with SRB stain. Excess stain was washed with acetic acid

density (O.D.) of each well was measured spectrophotometrically.

The IC50 values are the concentrations of thymoquinone required to produce 50% inhibition of cell growth. The experiment was repeated 3 times for each cell line.

3. Results and discussion

3.1. UV–visible spectra of metal chelates

UV–visible spectral data of the free LFX and glycine ligands and their ternary chelates (1 × 10–3 M) solutions in aqueous universal buffer of varying pH values ranged from 2 to 12 at λ ranging from 200 to 600 nm using the same solvent as blank are studied. It is obvious that free LFX ligand gives in the neutral and alkaline media two maximum bands, the first at 325 nm and another shoulder band at 295 nm. These bands may be attributed to n → π* and π → π* transitions inside the LFX molecule. But in the acidic medium the n → π* transition may be disappeared due to the protonation of nitrogen atom.

It is obvious from the absorption spectra obtained that all the ternary chelates have the same behavior. The band at 320 nm may be assigned to n → π* transition within the C=O group or carboxylate group of free LFX and glycine ligands. This band is red shifted to 321–326 nm in some chelates, while it disappeared in all the remaining chelates revealing the involvement of the C=O group of LFX ligand and carboxylate–O of amino acids in chelate formation [22,23].

The n → π* transition within the LFX molecule is overlapped with those of glycine molecule at λ = 320–350 nm so renders it difficult to attribute the blue or red shift of these bands as a result of the involvement of the N atom of LFX or amino group of glycine during chelate formation (N → M) [22,23].

3.2. Microanalysis

The elemental analyses data of the complexes (Table 1) show the formation of the complexes in the ratio 1:1:1 for [ML1L2]. It is found that the theoretical values are in agreement with the found values.

3.3. Molar conductance measurements

From conductivity measurements, performed in dimethylformamide, it is concluded from the results given in Table 1 that the Fe(III) and Cr(III) chelates have molar conductivity values of 178 and 152 Ω–1 cm–1 mol–1, respectively, indicating that they are 1:2 electrolytes. The molar conductivity values of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and UO2(II) chelates under investigation (Table 1) are found to be 108, 101, 126, 117, 124 and 139 Ω–1 cm–1 mol–1, respectively. It is obvious from these data that these chelates are ionic in nature and they are of the type 1:1 electrolytes. Th(IV) complex has a molar conductance value of 289 Ω–1 cm–1 mol–1, indicating its ionic nature and it is 1:3 electrolyte type.

3.4. IR spectral studies

The infrared spectra of various mixed ligand complexes synthesized are compiled in (Table 2 and Supplementary Fig. 2). The infrared spectra of these complexes in comparison with free LFX drug and the respective free glycine amino acid show characteristic band positions, shifts and intensities, which can be correlated to bidentate LFX drug and bidentate amino acid chelation. The IR spectra of the free LFX and glycine ligands and their metal complexes were carried out in the range of 4000–400 cm–1 and the most effective bands are listed in Table 2. The strong band observed at 1725 cm–1 in the free LFX ligand is assigned to the carbonyl stretching vibration [24–26]. This band is found in the spectra of the complexes at 1721–1739 cm–1 (Table 2) or disappeared in some complexes indicating the participation of carbonyl oxygen atom in coordination (M–O) [25]. The ν(OH), νasym(COO) and νsym(COO) stretching vibrations are observed at 3367, 1590 and 1396 cm–1, respectively, for LFX free ligand [27,28,25]. Coordination with metal ions via the carboxylate O atom indicated by shift in position of νasym(COO) and νsym(COO) stretching vibration bands to 1530–1590 and 1396–1475 cm–1, respectively, for LFX–Gly–metal complexes (Table 2). The ν(OH) band is still broad in all complexes, which renders it difficult to attribute to the involvement of OH group in coordination. In the 3400 cm–1 region,
one observes a broad absorption band due to the OH stretching of the water molecule, since, according to the elemental analysis and IR spectra (4000–400 cm\(^{-1}\)) one observes a broad absorption band due to the OH stretching of the water molecule, since, according to the elemental analysis and IR spectra (4000–400 cm\(^{-1}\)).

As regards, chelation through amino acids, the IR spectra exhibit significant features in the vNH\(_2\) and vCOO\(^-\) regions. It is worthwhile to mention here that free amino acids exist as zwitterions and the IR spectra of these cannot be compared entirely with those of metal complexes as amino acids in metal complexes do not exist as zwitterions. Free amino acids with NH\(_3\) functions in particular complexes, the IR spectra show characteristic bands in the region 3500–3300 cm\(^{-1}\) [29]. In the present complexes, the IR spectra show characteristic bands in the region 3428–3299 cm\(^{-1}\), which are lower compared to those of free vNH\(_2\). Hence, it can be concluded that the nitrogen of the amino group is involved in coordination. The IR spectra show strong evidence in support of the involvement of carboxylate group in coordination. In comparison with free amino acids, the vCOO\(^-\) (asym) and vCOO\(^-\) (sym) records different shifts, which confirm the monodentate [30,31] of the carboxylate group. The values of band shift \(\Delta\AA\) (v\(_{\text{asym}}\)COO\(^-\)–v\(_{\text{sym}}\)COO\(^-\)) are all about 143–205 cm\(^{-1}\), indicating that the carboxylate group in glycine is chelated in a uni-negatively coordinated manner to the metal ions.
Thus, it may be concluded that amino acids act as monobasic bidentates in these complexes coordinating through amino nitrogen and carboxylate oxygen [27–29]. Low intensity bands observed in far-IR region in the range 580–400 cm$^{-1}$ are due to $\nu$(M–O) and $\nu$(M–N) stretching vibrations [30,31]. The metal–oxygen stretching frequencies could not be assigned unambiguously due to the presence of three types of $\nu$(M–O) vibrations i.e., M–COO$^-$, M–H$\textsubscript{2}$O and M–C=O. However, the data are retained in Table 2 for clarity.

Therefore, from the IR spectral studies, it is concluded that LFX behaves as a neutral bidentate ligand with OO coordination sites and coordinated to the metal ions via the carbonyl oxygen and protonated carboxylic oxygen [24–28]. Glycine behaves as a uni-negative bidentate ligand with NO donor sites and coordinated to the metal ions via the amino group N and deprotonated carboxylic oxygen.

3.4.1. $^1$H NMR spectral studies

Unfortunately, the insolubility of glycine in DMSO-$d_6$ make it difficult to carry out $^1$H NMR spectrum of glycine to further clarify the way of binding of glycine ligand to the metal ions.

The proton NMR spectra of the LFX ligand and its diamagnetic Zn(II) complex were recorded in DMSO-$d_6$ solution using tetramethylsilane (TMS) as internal standard. The chemical shifts of the different types of protons of the LFX ligand and its diamagnetic Zn(II) complex are listed in Table 3 and represented graphically in Supplementary Fig. 3.

The comparative study of $^1$H NMR data of free LFX ligand and its zinc complex reveals about the ligatational behavior of the ligand. Upon comparison with the free LFX ligand, the signal observed at 13.5 ppm can be assigned to the carboxylate OH. This signal disappeared in the spectrum of the Zn(II) complex. Although the LFX ligand coordinated to the metal ions without proton displacement, the disappearance of this signal can be attributed to Zwitter-ion formation shown in Scheme 1 [19,24].

3.5. Magnetic moment measurements

The magnetic moment values are listed in Table 1. The magnetic moment values are found to be 3.55, 5.25, 5.82, 5.26, 2.97 and 2.03 BM for Cr(III), Mn(II), Fe(III), Co(II), Ni(II) and Cu(II) complexes, respectively, suggesting an octahedral geometry [23,32,33]. The Zn(II), UO$_2$(II) and Th(IV) complexes are diamagnetic. According to the empirical formulae of these complexes, an octahedral geometry was proposed.

3.6. Diffused reflectance spectral studies

A comparison of the electronic spectra of the free LFX drug and glycine ligands coordinated with those of the corresponding metal complexes, some shifts were detected. This can be considered as evidence for the complex formation. Additionally, the diffused reflectance spectra of metal complexes show different bands at different wavelengths, each one is corresponding to certain transition which suggests the geometry of the complexes. The diffused reflectance spectra of the complexes are dominated by intense intra-ligand charge transfer bands. The diffused reflectance spectrum of Cr(III) complex exhibits three bands at 27,411, 25,569 and 13,532 cm$^{-1}$ which may be assigned to the $^4$A$_{2g}$($F$) $\rightarrow$ $^4$T$_{2g}$($F$), $^4$A$_{2g}$($F$) $\rightarrow$ $^4$T$_{2g}$($F$) and $^4$A$_{2g}$($F$) $\rightarrow$ $^4$T$_{2g}$($P$) spin allowed $d$-$d$ transitions, respectively, suggesting an octahedral geometry [23,32,33]. The diffused reflectance spectrum of Mn(II) complex exhibits three bands at 25,506, 19,085 and 16,542 cm$^{-1}$, which may be assigned to $^4$T$_{1g}$ $\rightarrow$ $^6$A$_{1g}$, $^4$T$_{2g}$($G$) $\rightarrow$ 6A$_{1g}$ and $^4$T$_{2g}$($D$) $\rightarrow$ 6A$_{1g}$ transitions, respectively, which suggests an octahedral geometry [32,33]. From the diffused reflectance spectrum, it is observed that, the Fe(III) chelate

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shift, (d) ppm</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFX</td>
<td>13.5</td>
<td>(s, H, COOH)</td>
</tr>
<tr>
<td></td>
<td>8.93</td>
<td>(d, H, aromatic CH of carbon 5)</td>
</tr>
<tr>
<td></td>
<td>7.89</td>
<td>(d, 4H, CH$_2$ of carbon 5’, 5”)</td>
</tr>
<tr>
<td></td>
<td>3.57</td>
<td>(d, 2H, CH$_2$ of carbon 2’)</td>
</tr>
<tr>
<td></td>
<td>3.55</td>
<td>(d, CH of carbon 3’)</td>
</tr>
<tr>
<td></td>
<td>3.39</td>
<td>(d, 3H, CH$_2$ of carbon 1b)</td>
</tr>
<tr>
<td></td>
<td>1.46</td>
<td>(d, 3H, CH$_2$ of carbon 3a)</td>
</tr>
<tr>
<td></td>
<td>1.44</td>
<td>(d, H, CH of carbon 2)</td>
</tr>
<tr>
<td>[Zn(LFX)(Gly)(H$_2$O)$_2$]</td>
<td>8.87</td>
<td>(d, H, aromatic CH of carbon 5)</td>
</tr>
<tr>
<td></td>
<td>7.85</td>
<td>(d, 4H, CH$_2$ of carbon 5’, 5”)</td>
</tr>
<tr>
<td></td>
<td>3.41</td>
<td>(d, 2H, CH$_2$ of carbon 2’)</td>
</tr>
<tr>
<td></td>
<td>3.37</td>
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<td>3.02</td>
<td>(d, 3H, CH$_2$ of carbon 1b)</td>
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<tr>
<td></td>
<td>1.46</td>
<td>(d, 3H, CH$_2$ of carbon 3a)</td>
</tr>
</tbody>
</table>

Scheme 1. Zwitter-ion formation of LFX.

Table 3

$^1$H NMR spectral data of the lomefloxacin and its ternary chelates.
exhibits a band at 21,512 cm⁻¹, which may be assigned to the ³A₁g → ³T₁g(C) transition in octahedral geometry of the complex [23,32]. The ⁴A₁g → ³T₁g transition appears to be split into two bands at 12,845 and 15,758 cm⁻¹. The spectrum shows also a band at 28,089 cm⁻¹ which may be attributed to ligand to metal charge transfer. The diffused reflectance spectrum of the Co(II) complex displays three bands at 12,845 and 15,758 cm⁻¹ corresponding to the transitions ³T₁g(F) → ³T₂g(F) (v₁), ³T₁g(F) → ⁴A₂g(F) (v₂) and ³T₁g(F) → ⁴T₁g(P) (v₃), respectively, suggesting an octahedral geometry around Co(II) ion [23,32,33]. The region at 28,585 cm⁻¹ refers to the charge transfer band. The diffused reflectance spectrum of the Ni(II) complex displays three bands at 15,480, 12,723 and 14,492 cm⁻¹. The bands observed are assigned to the transitions ⁴T₁g(F) → ⁴T₂g(F) (v₁), ⁴T₁g(F) → ⁴A₂g(F) (v₂) and ⁴T₁g(F) → ⁴T₁g(P) (v₃), respectively, suggesting a monoclinic geometry of Ni(II) complex [23,32,33]. The region at 28,585 cm⁻¹ corresponds to a band at 27,024 cm⁻¹ which may be attributed to ligand to metal charge transfer.

The reflectance spectrum of the Cu(II) chelate consists of bands at 14,590 and 20,790 cm⁻¹ which can be attributed to the ²Eₙ and ²T₂ₕ transition normally observed for octahedral Cu(II) complexes [23,32]. An intense peak observed at 25,055 cm⁻¹ is due to ligand to metal charge transfer transition [32].

3.7. Thermal analyses

Thermogravimetric studies (TG) for the complexes were carried out within a temperature range from room temperature up to 800 °C. TG results are in a good agreement with the suggested formulae resulted from micranalyses data (Table 1). The determined temperature ranges and percent losses in mass of the solid complexes on heating are given in Table 4, which revealed the following findings:

The TG curve of LFX drug exhibits a first estimated mass loss of 45.02% (calcd. 44.98%) at 200–345 °C, which may be attributed to the liberation of C₁₀H₁₈N₂O₂ molecule as gases (Table 3). In the second estimated mass loss of 54.59% (calc. 54.94%), at 345–620 °C, which may be attributed to the liberation of C₁₂H₁₆F₂N₂O₃ molecule as gases.

The [Fe(LFX)(Gly)(H₂O)]Cl₂.H₂O complex gives a decomposition pattern as follows; the first stage is one step within the temperature range of 50–220 °C, representing the loss of H₂O (hydrated) with a found mass loss of 5.40% (calcd. 5.77%). The second stage represents the loss of two coordinated water and Cl₂ gases with a mass loss of 17.47% (calcd. 17.14%). The subsequent stage represents the loss of the remaining part of the complex with mass loss of 64.41% (calcd. 64.28%). At the end of the thermogram, the metal oxide Fe₂O₃ was the residue 12.27% (calcd. 13.02%), which is in good agreement with the calculated metal content obtained and the results of elemental analyses (Table 4).

The [Ni(LFX)(Gly)(H₂O)]Cl₂.H₂O complex is thermally decomposed in four stages. The first stage corresponds to a mass loss of 4.59% (calcd. 3.13%) within the temperature range 30–150 °C represents the loss of one molecule of hydrated water. The second stage corresponds to a mass loss of 24.56% (calcd. 25.50%) within the temperature range 150–250 °C and represents the loss of two coordinated water molecules, HCl and CH₂NO₂ with a found mass loss of 22.90% (calcd. 22.87%) within the temperature range 250–655 °C. The third and fourth stages, 255–695 °C, with a found mass loss of 49.48% (calcd. 50.48%) is reasonably accounted for decomposition of the remaining organic part of the complex leaving out NiO as a residue with a found mass loss of 21.01% (calcd. 20.89%).

The [Cu(LFX)(Gly)(H₂O)]Cl₂.H₂O complex is thermally decomposed in four stages. The first stage corresponds to a mass loss of 3.69% (calcd. 3.11%) within the temperature range 115–255 °C represents the loss of one molecule of hydrated water. The second stage corresponds to a mass loss of 21.01% (calcd. 20.89%) within the temperature range 220–275 °C and represents the loss of two coordinated water molecules, HCl and CH₂NO₂ with a found mass loss of 5.40% (calcd. 5.77%). The second step represents the loss of two coordinated water and Cl₂ gases with a mass loss of 49.48% (calcd. 50.48%) is reasonably accounted for decomposition of the remaining organic part of the complex leaving out CuO as a residue with a found mass loss of 21.01% (calcd. 20.89%).
NO$_3$ and C$_6$H$_{10}$F$_2$O$_4$ gases with an estimated mass loss of 32.13% (calcld. 33.04%) occurs. The second step within the temperature range 340–650 °C, involves liberation of C$_4$H$_2$A$_2$N$_2$O molecule with an estimated mass loss of 25.10% (calcld. 24.56%).

3.8. Kinetic data

The kinetic and thermodynamic parameters of thermal degradation process have been calculated using Coats–Redfern and Horowitz–Metzger models [34,35]. Coats–Redfern relation [34] is as follows:

$$\ln(\ln(1 - x)/T^2) = \ln(AR)/\beta E - (E/RT)$$

(1)

$x$ represents the fraction of sample decomposed at time $t$, defined by: $x = (w_n - w_t)/(w_n - w_\infty)$, where $w_n$, $w_t$, and $w_\infty$ are the weight of the sample before the degradation, at temperature $T$ and after total conversion, respectively. $T$ is the derivative peak temperature. $\beta$ is the heating rate = $dT/dt$. $E$ and $A$ are the activation energy and the Arrhenius pre-exponential factor, respectively. A plot of $\ln[−(ln(1 − x))/T^2]$ versus $1/T$ gives a straight line whose slope ($E/RT$) and the pre-exponential factor ($A$) can be determined from the intercept.

The Horowitz–Metzger equation [35] is an illustrative of the approximation methods. These authors derived the relation:

$$\log[(1 - (1 - x)^{1-n})/(1 - n)] = E/\Phi/2.303RT^2$$

(2)

when $n = 1$, the LHS of Eq. (2) would be $\log[-\log(1 - x)].$ For a first order kinetic process the Horowitz–Metzger equation may be written in the form (Eq. (3)):

$$\log[\log(w_n/w_t)] = E/\Phi/2.303RT^2 - \log 2.303$$

(3)

where $\theta = T - TS$, $w_t = w_\infty - w$, $w_\infty = mass$ loss at the completion of the reaction; $w = mass$ loss up to time $t$. The plot of $\log[\log(w_n/w_t)]$ versus $\theta$ was drawn and found to be linear from the slope of which $E$ was calculated. The pre-exponential factor, $A$, was calculated from the Eq. (4):

$$E/RT^2 = A/[\phi \exp(-E/RT)]$$

(4)

A number of pyrolysis processes can be represented as a first order reaction. The degradation of a series LFX complexes was suggested to be first order, therefore we assume $n = 1$ for the remainder of the present text. The other thermodynamic parameters such as activation energy ($E$), preexponential factor ($A$), entropy of activation ($\Delta S$), enthalpy of activation ($\Delta H$) and free energy of activation ($\Delta G$) of decomposition steps were calculated using Coats–Redfern [34] and Horowitz–Metzger [35] methods and the average values obtained are listed in Table 5. From the results, the following remarks can be pointed out:

- The high values of the energy of activation of the complexes reveal the high stability of such chelates due to their covalent bond character [36].
- The positive sign of $\Delta G$ for the investigated complexes reveals that the free energy of the final residue is higher than that of the initial compound, and all the decomposition steps are non-spontaneous processes. Also, the values of the activation, $\Delta G$ increases significantly for the subsequent decomposition stages of a given complex. This is due to increasing the values of $TAS$ significantly from one step to another which overrides the values of $\Delta H$ [36].

The negative values of $\Delta S$ for all the complexes indicate more ordered activated complex than the reactants or the reaction is slow [36].

The data listed in Table 5 show that the activation energy $E$ change with the increase of atomic number in the 3rd series of d-block elements, for the first decomposition step of their complexes. The complexes of Fe(III) ($d^5$) and Ni(II) ($d^8$) present higher that of Cu(II) ($d^9$) complex. This means that these complexes are more stable at the beginning of the decomposition than those on the Cu(II) complex [37]. The same trend is gained for these complexes at the second steps of decomposition, except they become excited before decomposition. These quantitative explanations of results mean also that the thermal stability is mainly related to the stereo-structure of the complexes and the electronic configuration of the d-block elements. Therefore, pairing up energy $P$ and energy of splitting of metal orbitals in octahedral $A_0$, in strong environment are calculated. It is obvious that, those elements of Fe(III) ($d^5$) ($t_2g^2e_g$, $A_0$ = –10/12 – $P$) and Ni(II) ($d^8$) ($t_2g^6e_g^2$, $A_0$ = –6/12 – $2P$) are stable since they have high pairing up energy and even an electron system. The Cu(II) ($d^9$) ($t_2g^6e_g^2$, $A_0$ = 3/12) has less stability due to the lower pairing effect. It can be concluded that, the relation between $E$ vs. atomic number gives a quantitative interpretation to the thermal stability of the complexes [37].

3.9. X-ray powder diffraction

X-ray powder diffraction pattern in the $0^\circ < 2\theta < 60^\circ$ of the LFX and glycine ligands and their metal complexes were carried out in order to give an insight about the lattice dynamics of these complexes. The X-ray powder diffraction obtained reflects a shadow on the fact that each solid represents a definite compound of a definite structure which is not contaminated with starting materials. XRD analysis for ternary chelates shows that the coordination of LFX alone or in the presence of glycine to the metal ions changes the XRD pattern of the ligands. This means that the metal ions are not fitted in the same phase of LFX and glycine. Therefore, the non-similarity of XRD pattern between the metal ions and ternary chelates suggests that these chelates have a different phase
structures than the LFX and glycine ligands. In addition, on comparing the XRD spectra of the ternary chelates with the XRD spectra of the free ligands, indicate the crystallinity of the ternary chelates under study, except \([\text{Co(LFX)(Gly)(H}_2\text{O)}_2]\)\(_2\)Cl and \([\text{UO}_2\text{(LFX)(Gly)}](\text{NO}_3)\) chelates, they can be considered as amorphous structure.

4. Structural interpretation

From all of the above observations, the structure of these complexes may be interpreted in accordance with ternary complexes of LFX drug and glycine with a similar distribution of like coordinating sites\([19,24,27,28]\). The structural information from the previously reported complexes is in agreement with the data reported in this paper based on the IR, molar conductivity, magnetic and electronic spectra measurements. Consequently, the structures proposed are based on octahedral geometry. The LFX drug always coordinates via the carbonyl O and the protonated O atoms forming a two binding chelating site and glycine behave as uninegative bidentate ligand with NO donor sites and coordinated to the metal ions via the amino group N and deprotonated carboxylic oxygen. The proposed general structures are shown in Fig. 4.

5. Biological activity

5.1. Antimicrobial activity

Metal complexes may affect living systems. This has been known for over a hundred years in respect of the curare-like activity in mammals of some metal amines. The antibacterial activities of transition metal complexes have been extensively studied following the discovery that chelating agents inhibit growth of bacteria when complexed with many metals. To assess the biological potential of the synthesized compounds, the LFX and glycine ligands and their mixed metal complexes was tested against the selected bacteria strains. The antimicrobial data were collected in Table 6. The synthesized complexes were found to possess remarkable bactericidal properties, it is however interesting that the biological activity gets enhanced on undergoing complexation with the metal ions. Finally, to complete the evaluation of biological activity of the synthesized complexes, some comparison with the known standard antibiotic were performed and the data are collected in Table 6 and shown in Fig. 5 and Supplementary Fig. 6.

The biological activity against the two gram positive (Staphylococcus aureus and Bacillus subtilis) and gram negative bacteria (Escherichia coli and Neisseria gonorrhoeae) of the ternary metal

<table>
<thead>
<tr>
<th>Sample</th>
<th>Inhibition zone diameter (mm/mg sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>Control: DMSO</td>
<td>0.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>10</td>
</tr>
<tr>
<td>LFX</td>
<td>43</td>
</tr>
<tr>
<td>([\text{Cr(LFX)(Gly)(H}_2\text{O)}_2])(_2)Cl</td>
<td>42</td>
</tr>
<tr>
<td>([\text{Mn(LFX)(Gly)(H}_2\text{O)}_2])(_2)Cl</td>
<td>44</td>
</tr>
<tr>
<td>([\text{Fe(LFX)(Gly)(H}_2\text{O)}_2])(_2)Cl</td>
<td>45</td>
</tr>
<tr>
<td>([\text{Ni(LFX)(Gly)(H}_2\text{O)}_2])(_2)Cl</td>
<td>40</td>
</tr>
<tr>
<td>([\text{Co(LFX)(Gly)(H}_2\text{O)}_2])(_2)Cl</td>
<td>40</td>
</tr>
<tr>
<td>([\text{Zn(LFX)(Gly)(H}_2\text{O)}_2])(_2)Cl</td>
<td>46</td>
</tr>
<tr>
<td>([\text{Th(LFX)(Gly)(H}_2\text{O)}_2])(_2)Cl</td>
<td>43</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BNO%7D_3">\text{UO}_2\text{(LFX)(Gly)}</a>)(_2)H(_2)O</td>
<td>49</td>
</tr>
</tbody>
</table>

Standard Tetracycline

Order of activity: Tetracycline > Zn(II) > Co(II) > Fe(III) > Th(IV) > Fe(III) > Co(II) > Mn(II) > Ni(II) > Cr(III) > tetracycline > glycine

56. Biological activity

5.1. Antimicrobial activity

Metal complexes may affect living systems. This has been known for over a hundred years in respect of the curare-like activity in mammals of some metal amines. The antibacterial activities of transition metal complexes have been extensively studied following the discovery that chelating agents inhibit growth of bacteria when complexed with many metals. To assess the biological potential of the synthesized compounds, the LFX and glycine ligands and their mixed metal complexes was tested against the selected bacteria strains. The antimicrobial data were collected in Table 6. The synthesized complexes were found to possess remarkable bactericidal properties, it is however interesting that the biological activity gets enhanced on undergoing complexation with the metal ions. Finally, to complete the evaluation of biological activity of the synthesized complexes, some comparison with the known standard antibiotic were performed and the data are collected in Table 6 and shown in Fig. 5 and Supplementary Fig. 6.

The biological activity against the two gram positive (Staphylococcus aureus and Bacillus subtilis) and gram negative bacteria (Escherichia coli and Neisseria gonorrhoeae) of the ternary metal
complexes is higher than that of the LFX drug and glycine ligands and tetracycline standard. It is found that the activity increases upon coordination. The increased activity of the metal chelates can be explained on the basis of chelation theory [38]. The orbital of each metal ion is made so as to overlap with the ligand orbital. Increased activity enhances the lipophilicity of complexes due to delocalization of π-electrons in the chelate ring [39]. In some cases increased lipophilicity leads to breakdown of the permeability barrier of the cell [38,39].

In Table 6, it is clear that although both of [Mn (LFX) (Gly) (H₂O)₂]Cl and [Zn (LFX) (Gly) (H₂O)₂]Cl complexes are nearly similar (except difference in ions where both of ions have II valance state), the inhibition zone diameter for B. subtilis, S. aureus, N. gonorrhoeae and E. coli are different. The antimicrobial mechanism of zinc complexes involves the capability of zinc ions to inhibit glycolysis of microorganisms by oxidizing thiols groups in essential glycolytic enzymes. It is therefore suggested that the potentiation of bacterial killing activity of Zn complex may be due to facilitation of entry of zinc ions into the inside of bacterial cells [40]. In addition, in biological systems, only manganese ion is readily capable of replacing Mg(II), but only in a limited set of circumstances. Mn(II) effectively binds ATP and allows hydrolysis of the energy molecule by most

Table 7

<table>
<thead>
<tr>
<th>Complex</th>
<th>Surviving fraction (MCF7)</th>
<th>IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (µg/ml)</td>
<td>0.0</td>
</tr>
<tr>
<td>LFX</td>
<td>1.00</td>
<td>0.64</td>
</tr>
<tr>
<td>[Mn(LFX)(Gly)(H₂O)₂]Cl</td>
<td>1.00</td>
<td>0.99</td>
</tr>
<tr>
<td>[Co(LFX)(Gly)(H₂O)₂]Cl</td>
<td>1.00</td>
<td>0.59</td>
</tr>
<tr>
<td>[Ni(LFX)(Gly)(H₂O)₂Cl]H₂O</td>
<td>1.00</td>
<td>0.99</td>
</tr>
<tr>
<td>[Cu(LFX)(Gly)(H₂O)₂Cl]H₂O</td>
<td>1.00</td>
<td>0.99</td>
</tr>
<tr>
<td>[Zn(LFX)(Gly)(H₂O)₂]Cl</td>
<td>1.00</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Fig. 5. Biological activity of LFX, Gly and ternary complexes.

Fig. 7. Anticancer activity of LFX and its ternary complexes.
ATPases. Mn(II) can also replace Mg(II) as the activating ion for a number of Mg(II)-dependent enzymes, although some enzyme activity is usually lost [40]. Sometimes such enzyme metal preferences vary among closely related species: for example, the reverse transcriptase enzyme of lentiviruses like HIV, SIV and FIV is typically dependent on Mg(II), whereas the analogous enzyme for other retroviruses prefers Mn(II) [41]. Also according to the chelation theory [38], the difference in activity between the two metal ions (Mn(II) had d^5 and Zn(II) had d^{10} configurations) can be attributed to the difference in probability of the d orbitals to overlap with the ligand orbitals. Also it could be due to the difference in electronegativity, size of the metal ion and the intermediate complex formation.

5.2. Cytotoxic activity

The lomefloxacin drug, glycine and all their ternary metal complexes were tested for their activity against breast cancer cell line (MCF7) by using 100 μg/ml drug concentration. From these results, it is clear that LFX, [Mn(LFX)(Gly)(H2O)2]Cl, [Co(LFX)(Gly)(H2O)2]Cl, [Ni(LFX)(Gly)(H2O)2]Cl2.H2O, [Cu(LFX)(Gly)(H2O)2]Cl2.H2O and [Zn(LFX)(Gly)(H2O)2]Cl were found to be very active against breast cancer cells with inhibition ratio values between 70% and 85.5%, while other complexes utilized in this work had been shown to be inactive (less than 70% inhibition). It is clear that a pattern of activity can be determined using different drug concentrations (Table 7). LFX, [Mn(LFX)(Gly)(H2O)2]Cl, [Co(LFX)(Gly)(H2O)2]Cl and [Zn(LFX)(Gly)(H2O)2]Cl were found to be very active with IC50 values 14, 11.2, 13 and 16.8, respectively, while glycine and other complexes had been shown to be inactive at lower concentration than 100 μg/ml (Fig. 7).

6. Conclusion

The lomefloxacin drug (LFX) and its transition metal complexes with glycine have been prepared and fully characterized using the different spectral and physicochemical studies. The LFX drug has several coordinating sites which are suitably spatially arranged to bind transition metals which require hexa-coordination and glycine as essential amino acid behaves as a uni-negative bidentate ligand with NO donor sites and coordinated to the metal ions via the amino group N and deprotonated carboxylic oxygen. Significantly, further investigations on their antitumor activity revealed that such complexes possessed a considerable antitumor activity which was thought to be related with its DNA-binding affinity. The better binding properties of the complexes should be attributed to the good coplanarity of the ligand after coordination with metal ions. Meanwhile, nature of the central metal ions also affected the intercalative ability. Further study of complexes based on LFX as antibacterial drug is ongoing in our group.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.molstruc.2011.05.018.

References