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Thermal degradation behavior of some ruthenium complexes with fluoroquinolone derivatives as potential antitumor agents

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Abstract Three new complexes with ligands belong to the fluoroquinolone class having the general formula $[RuL_2Cl_2]Cl\ nH_2O$ ((**1**) L: norfloxacin (nf), $n = 4$; (**2**) L: ciprofloxacin (cp), $n = 3$; (**3**) L: enrofloxacin (enro), $n = 5$) were synthesized and characterized by chemical analysis UV–Vis and IR spectroscopy. In all complexes fluoroquinolone derivative acts as bidentate chelate ligand. The thermal behavior steps were investigated in synthetic air flow. The thermal transformations are complex processes according to TG and DTG curves including dehydration, quinolone derivative degradation, as well as $RuCl_3$ conversion in RuO_2.

Keywords Fluoroquinolone · Ruthenium complexes · Thermal behavior

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Introduction

Quinolones belong to a class of synthetic antibacterial agents active that act as specific inhibitors of two essential bacterial enzymes, DNA gyrase (topoisomerase II) and DNA topoisomerase IV [1–3]. Due to their specific mode of action, they are considered to be the broadspectrum antibiotics active against gram-positive and gram-negative pathogens. The first quinolone antibiotic was nalidixic acid, introduced as antibacterial agent in the 1960s [4]. This first generation of quinolone was active against gram-negative bacteria, and the use of this drug was essentially limited to urinary tract infections. The chemical modification of the basic structure of nalidixic acid resulted in a second generation of quinolones. They possess a carboxylic group in position 3 and a carbonyl group in position 4, being often referred to as 4-quinolones. Addition of 6-fluoro and 7-piperazinyl groups to this basic structure leads to the group of compounds generally known as the fluoroquinolones greatly effective against both gram-negative and gram-positive pathogens that are resistant to other antibacterials [5].

Norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazin-1-yl-quinoline-3-carboxylic) nf (Fig. 1a), ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazin-1-yl-quinoline-3-carboxylic acid), cp (Fig. 1b), and enrofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-ethyl-piperazin-1-yl-quinoline-3-carboxylic acid) enro (Fig. 1c) are typical second-generation fluoroquinolone drugs. Norfloxacin are used in uncomplicated urinary tract infections [6], while ciprofloxacin are used in complicated urinary tract infections, gastroenteritis with severe diarrhea prostatitis and nosocomial infections, sexually transmitted diseases, anthrax [7]. Enrofloxacin is the first fluoroquinolone developed for veterinary application in

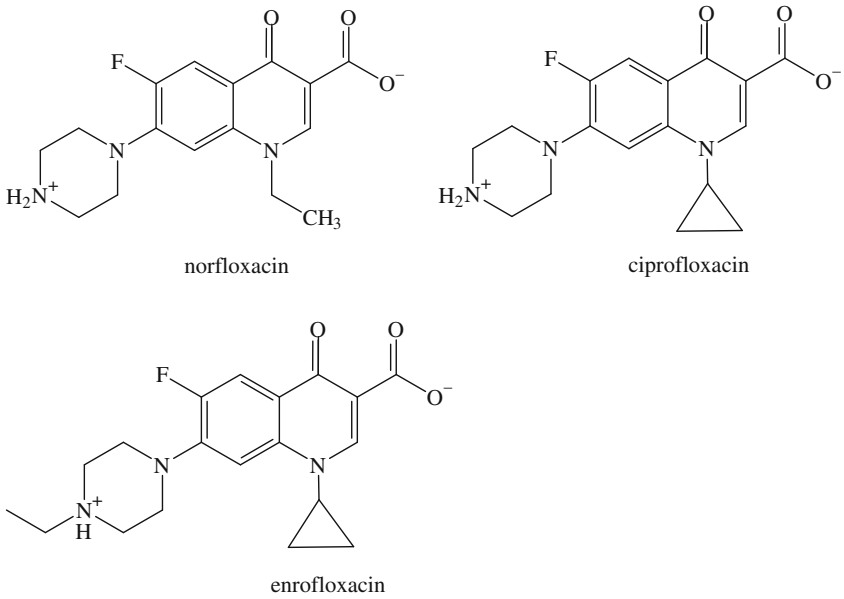

Fig. 1 Structure of fluoroquinolone ligands

treatment of uncomplicated and complicated urinary tract infections, pyelonephritis, sexually transmitted diseases, prostatitis, skin and tissue infections, and urethral and cervical gonococcal infections [8–10]. The neutral quinolones in the zwitterionic state (Fig. 1) are capable of forming simple complexes. In these complexes the quinolone coordinate to metal ions as a bidentate ligand via the ring carbonyl group at position 4 and through one of the oxygen atoms of the carboxylate group at position 3. In these complexes, the divalent cations like Mg(II), Ca(II), Ba(II) [11], Mn(II), Co(II) [12, 13], Pd(II) [14], Cu(II) [13, 15], Zn(II), Cd(II), Hg(II) [16, 17] are preferred. As from trivalent cations only a few reports describe complexes with Fe(III) [12, 18]. Another trivalent cation which forms complexes with biological relevance is Ru(III). Ruthenium(III) complexes are very promising antitumor agent, especially from the point of view of overcoming cisplatin resistance with a low general toxicity. Most of these complexes contain nitrogen-donor ligands. Ammine-chlorido derivatives like *cis*-$[Ru(III)(NH_3)_4Cl_2]^+$ and especially *fac*-$[Ru(III)(NH_3)_3Cl_3]$ display a comparable antitumor activity to that of cisplatin in a few selected cell lines [19, 20]. Na{*trans*-[Ru(III)Cl_4(dmso)(Him)]}, (Him = imidazole), nicknamed NAMI, and the more stable $[H_2Im]$[*trans*-Ru(III)Cl_4(dmso)(Him)], also known as NAMI-A are very promising dimethylsulfoxide complex. A series of NAMI-A analogs bearing a weakly basic heterocyclic nitrogen ligand *trans*- to dmso was synthesized [21]. Anionic ruthenium(III) complexes with other heterocyclic nitrogen ligands have been synthesized [22]. The most successful of which have the formula *trans*-$[RuCl_4(L)_2]^-$, where L is imidazole (KP418) or indazole (KP1019 and KP1339), and the counterion $(LH)^+$ or Na^+. KP1019 completed phase-I clinical trials [23]. Ruthenium polyaminocarboxylate complexes represent a class of ruthenium(III) complexes with polydentate mixed-donor ligands from the H_4 edta family. These complexes are interesting from their acid–base and redox properties [24–27].

A systematic study to examine the interaction between quinolone derivatives and ruthenium(III) in different reaction media has been initiated [28, 29].

The aim of this study was to synthesize, in solid state, the complexes of Ru(III) with norfloxacin, ciprofloxacin, and enrofloxacin coordinated through two oxygen donors from carbonyl group and carboxylate group.

The elemental analysis, infrared, electronic spectra, and thermal decomposition were performed in order to elucidate the coordination properties of fluoroquinolones investigated. The thermal curves provided suggestions about the composition and the number and nature of the solvent molecules also.

Experimental

All chemicals were purchased from Sigma-Aldrich, reagent grade and were used without further purification.

Synthesis

The quinolone ligands are insoluble in water, but are slowly solubilized in slightly acidic media created by the Ru(III) chloride. Total solubilization is achieved for a metal:ligand molar ratio of 1:2.

A quantity equivalent with 0.8 mmol ligand (0.226 g norfloxacin, 0.265 g ciprofloxacin and 0.288 g enrofloxacin, respectively) was suspended in water (30 mL) and 0.4 mmol of ruthenium(III) chloride monohydrate (0.0902 g) was added while stirring. The final pH of the resulting brown solution was 5. The solution was kept on the steam bath until a fivefold reduction in volume has been achieved. The brown precipitate, which formed after the addition of an equal volume of ethanol, was collected by filtration, washed with ethanol, and dried in air.

Chemical analyses and physical measurements

The chemical analyses were performed on a Perkin Elmer PE 2400 analyzer (for C, H, N, S) and a Shimadzu AA-6300 spectrometer (for Ru).

IR spectra were recorded in KBr pellets with an FT-IR VERTEX 70 (Bruker) spectrometer in the range 400–4000 cm^{-1}.

Electronic spectra by diffuse reflectance technique, with Spectralon as standard, were recorded in the range 200–1,000 nm, on a Jasco V 670 spectrophotometer.

Complex $[Ru(nf)_2Cl_2]Cl{\cdot}4H_2O$ (**1**): Analysis, found: Ru, 10.86; C, 41.41; H, 5.05; N, 9.13%; calculated for $RuC_{32}H_{44}F_2N_6O_{10}Cl_3$: Ru, 11.00; C, 41.86; H, 4.83; N, 9.15%; IR (KBr pellet), cm^{-1}: $\nu(C{=}O)_p$, 1716 m; $\nu_{as}(OCO)$, 1630 vs; $\nu_s(OCO)$, 1396 m.

Complex $[Ru(cp)_2Cl_2]Cl{\cdot}3H_2O$ (**2**): Analysis, found: Ru, 10.81; C, 44.28; H, 4.67; N, 8.94%; calculated for $RuC_{34}H_{42}F_2N_6O_9Cl_3$: Ru, 10.94; C, 44.19; H, 4.58; N, 9.09%; IR (KBr pellet), cm^{-1}: $\nu(C{=}O)_p$, 1720 m; $\nu_{as}(OCO)$, 1629 vs; $\nu_s(OCO)$, 1384 m.

Complex $[Ru(enro)_2Cl_2]Cl{\cdot}5H_2O$ (**3**): Analysis, found: Ru, 9.31; C, 44.97; H, 5.31; N, 8.12%; calculated for $RuC_{38}H_{54}F_2N_6O_{11}Cl_3$: Ru, 9.94; C, 44.91; H, 5.36; N, 8.27%; IR (KBr pellet), cm^{-1}: $\nu(C=O)_p$, 1707 s; $\nu_{as}(OCO)$, 1623 vs; $\nu_s(OCO)$, 1404 m.

The heating curves (TG and DTA) were recorded using a Labsys 1200 SETARAM instrument, with a sample mass of 15–25 mg over the temperature range 30–900 °C, using a heating rate of 10 K/min. The measurements were carried out in synthetic air (flow rate 16.66 cm^3/min) by using alumina crucibles.

Results and discussion

Physico-chemical characterization of complexes

The major aim of this study was to synthesize complexes of some complexes with fluoroquinolone derivatives (Fig. 1) coordinated, in zwitterionic form, through oxygen atoms. The complexes that could behave as anticancer agents were characterized by analytical and physico-chemical analyses as well as the thermogravimetric investigation.

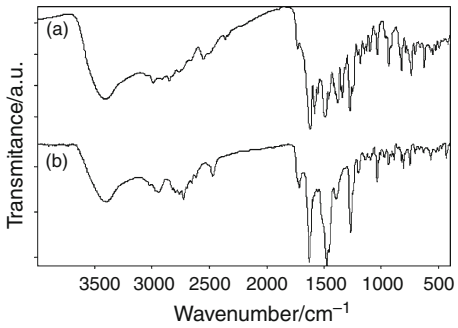

Fig. 2 IR spectra for norfloxacin (nf) (**a**) and $[Ru(nf)_2Cl_2]Cl{\cdot}4H_2O$ (**b**)

Table 1 Electronic spectral data for ruthenium–fluoroquinolone complexes (nm)

Complex	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	$^2T_{2g} \rightarrow {}^2A_2, {}^2T_1$	$^2T_{2g} \rightarrow {}^4T_{1g}$
(**1**)	265	340	375	420
				495
(**2**)	265	330	380	455
				500
(**3**)	290	335	385	420
				495

On the basis of the above data the proposed coordination for the complexes is as follows (Fig. 4)

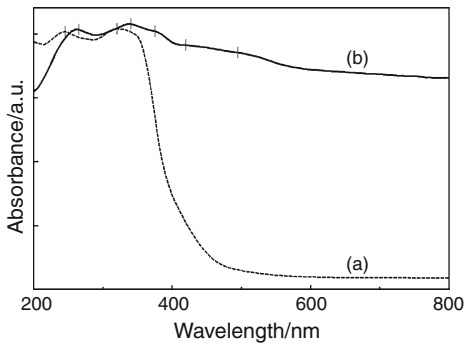

Fig. 3 Electronic spectra for norfloxacin (nf) (**a**) and $[Ru(nf)_2Cl_2]Cl{\cdot}4H_2O$ (**b**)

The complexes have been formulated on the basis of chemical analyses UV–Vis and IR spectra as follows:

$$\left[Ru(nf)_2Cl_2\right]Cl \cdot 4H_2O \quad (1)$$

$$\left[Ru(cp)_2Cl_2\right]Cl \cdot 3H_2O \quad (2)$$

$$\left[Ru(enro)_2Cl_2\right]Cl \cdot 5H_2O \quad (3)$$

The IR spectra of complexes show the characteristic patterns of quinolone derivatives, which act as bidentate

chelate (Fig. 2a, b). The characteristic bands assigned to the carboxylato group, ν_{as}(OCO) and ν_s(OCO), appear around 1620 and 1390 cm^{-1}, respectively. The separation

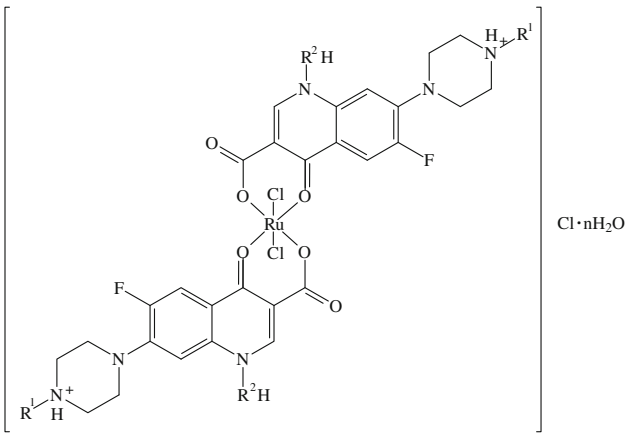

Fig. 4 The proposed formula for ruthenium–fluoroquinolone complexes

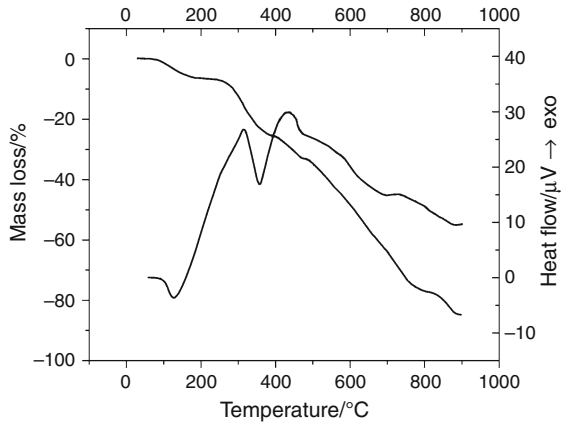

Fig. 5 TG and DTA curves for the thermal decomposition of $[Ru(nf)_2Cl_2]Cl{\cdot}4H_2O$

Δ between these bands, greater than 200 cm^{-1}, suggests a unidentate coordination mode [30]. The complexes show a group of weak and medium intensity bands in the range 2850–3450 cm^{-1}. These bands can be assigned to the vibration of the quaternary amine group from piperazine moiety together with methylene groups [12] indicating clearly the zwitterionic form of ligands.

Electronic spectra of complexes and corresponding ligands were recorded (Table 1). Spectra of the complexes display ligand bands shifted to lower energies as a consequence of the electronic modifications suffered by the ligands during the interaction with the metallic ion. In addition, the spectra of complexes contain bands characteristic to the spin allowed and forbidden transition for Ru(III) $4d^5$ low spin configuration in an octahedral stereochemistry (Fig. 3) [31].

Thermal behavior of complexes

An analysis of the thermal degradation behavior of the ruthenium–fluoroquinolone complexes was the primary focus of the study reported here. Confirmation of composition and an assessment of the role of solvent in complex formation were of major interest (Fig. 4).

Thermal decomposition of $[Ru(nf)_2Cl_2]Cl{\cdot}4H_2O$

The TG and DTA curves corresponding to decomposition of complex (**1**) are displayed in Fig. 5. These plots suggest that decomposition occurs in at least three major steps.

The first step corresponds to an endothermic volatilization of water (Table 2). The second step does not represent a single process but rather is reflective of two overlapping processes and probably corresponds to partial oxidative degradation of the norfloxacin ligand. The oxidative removal of ligand side groups has been observed for

Table 2 Thermal decomposition data for ruthenium–fluoroquinolone complexes

Complex	Step	Thermal effect	Temperature range/°C	Δm_{exp}/%	Δm_{calc}/%
$[Ru(nf)_2Cl_2]Cl{\cdot}4H_2O$	1	Endothermic	70–180	7.7	7.8
	2	Exothermic	230–480	24.2	24.0
	3	Exothermic	480–790	45.3	45.6
	4	Exothermic	790–900	8.2	8.1
$[Ru(cp)_2Cl_2]Cl{\cdot}3H_2O$	1	Endothermic	70–130	5.6	5.8
	2	Exothermic	150–340	26.5	26.4
	3	Exothermic	340–730	45.5	45.3
	4	Exothermic	730–900	7.9	8.1
$[Ru(enro)_2Cl_2]Cl{\cdot}5H_2O$	1	Endothermic	70–160	8.8	8.9
	2	Exothermic	175–520	30.1	29.9
	3	Exothermic	520–780	40.5	40.8
	4	Exothermic	780–900	7.4	7.3

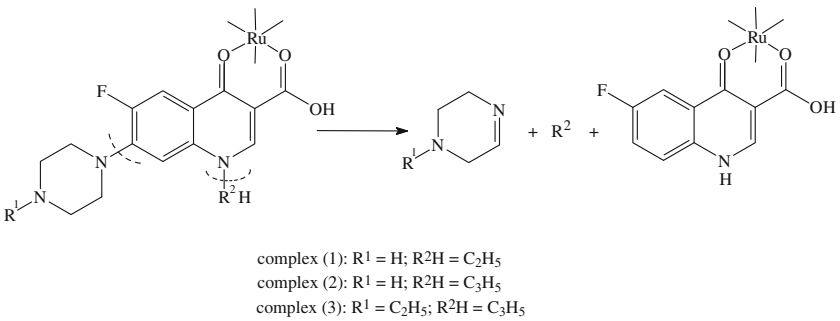

Fig. 6 Proposed degradation mode of ruthenium–fluoroquinolone complexes

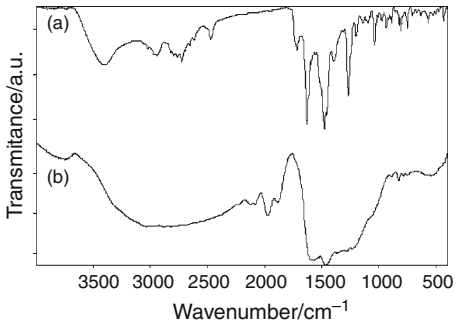

Fig. 7 IR spectra for $[Ru(nf)_2Cl_2]Cl{\cdot}4H_2O$ (**a**) and residue at 480 °C

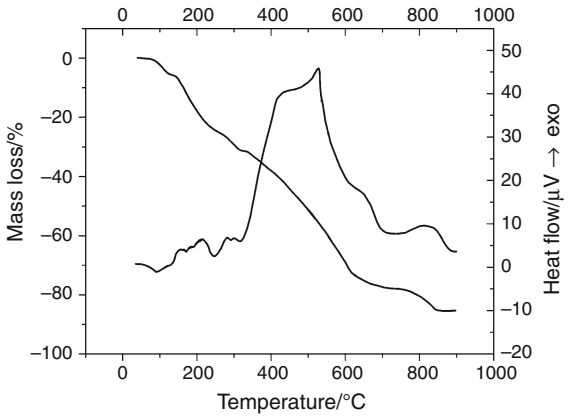

Fig. 8 TG and DTA curves for the thermal decomposition of $[Ru(cp)_2Cl_2]Cl{\cdot}3H_2O$

other norfloxacin complexes of similar structure (Fig 6) [32]. The IR spectrum of residue at 480 °C sustains this assumption, the characteristic bands of the carboxylic and carbonylic groups still appear in the IR spectrum of this intermediate (Fig. 7b) while the characteristic bands of the methylene and quaternary ammine group are absent in this spectrum

Next step, exothermic also corresponds to organic part loss according to TG curve. The resulted intermediate, $RuCl_3$, turns in RuO_2 in the last step, process accompanied by an exothermic effect (found/calcd. overall mass loss: 91.8/91.9).

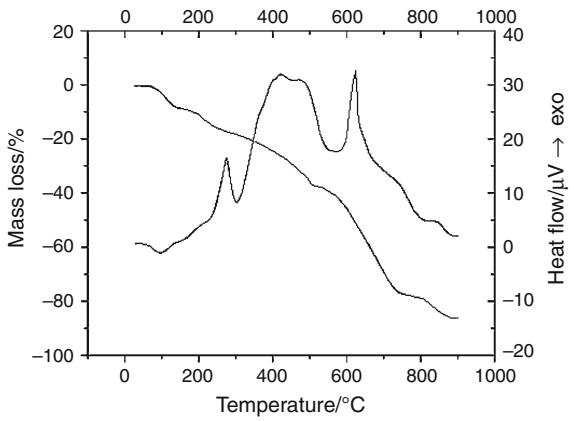

Fig. 9 TG and DTA curves for the thermal decomposition of $[Ru(enro)_2Cl_2]Cl{\cdot}5H_2O$

Thermal decomposition of $[Ru(cp)_2Cl_2]Cl{\cdot}3H_2O$

Complex (**2**) loses the water molecules in the 70–130 °C range (Fig. 8). The thermal degradation of ciprofloxacin occurs in at least two successive processes as both TG and DTA indicate. In the third step the complex intermediate leads to $RuCl_3$. The remaining $RuCl_3$ generates RuO_2 but up to 1000 °C this transformation is not finished (found/calcd. overall mass loss: 92.1/91.9).

Thermal decomposition of $[Ru(enro)_2Cl_2]Cl{\cdot}5H_2O$

The decomposition of complex (**3**) comprises also four steps and starts with water elimination, (Fig. 9). The anhydrous species starts to decompose at 175 °C following the same pattern. After the enrofloxacin oxidative degradation, the ruthenium chloride leads to RuO_2 in the final step according to the mass variation (found/calcd. overall mass loss: 92.6/92.7).

Conclusions

The new complexes of Ru(III) fluoroquinolone as ligands belong to a class of coordination compounds of current

interest having in view the cytostatic effect evidenced for similar species.

For all complexes fluoroquinolone derivative acts as bidentate chelate according to IR data.

Thermal analysis (TG, DTA) of these complexes elucidated the composition and also the number and nature of water molecules. It was also evidenced the existence of an intermediate step corresponding to the elimination of side groups for all compounds. The final product is ruthenium(IV) oxide.

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