

IN VITRO STUDIES REGARDING THE INTERACTIONS OF SOME NOVEL RUTHENIUM (III) COMPLEXES WITH DOUBLE STRANDED CALF THYMUS DEOXYRIBONUCLEIC ACID (DNA)

ANDREEA LETIȚIA ARSENE¹, VALENTINA UIVAROSI², NICULINA MITREA³, CRISTINA MANUELA DRĂGOI^{3*}, ALINA CRENGUȚA NICOLAE³

¹Department of Microbiology, Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davila", 6, Traian Vuia Str, sector 2, Bucharest, Romania

²Department of General and Inorganic Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davila", 6, Traian Vuia Str, sector 2, Bucharest, Romania

³Department of Biochemistry, Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davila", 6, Traian Vuia Str, sector 2, Bucharest, Romania

*corresponding author: manuela.dragoi@gmail.com

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Abstract

Following the success of cisplatin, several metal complexes other than platinum have been considered over the years as possible alternatives to cisplatin; particularly it was found that ruthenium (III) compounds possess antitumour and antimetastatic activities. The literature studies revealed the affinity of these complexes for crucial biomolecules like DNA and provide evidence for formation of stable adducts therewith. The current paper presents the DNA-binding properties of some ruthenium (III) complexes with quinolones and dimethylsulfoxide, with the general formula $RuCl_3L_2(DMSO)_m \cdot nH_2O$ (L: pipemidic acid (pip), $m = 1, n = 2$ (**Ru-pip**); L: enoxacin (enx), $m = 1, n = 0$ (**Ru-enx**); L: norfloxacin (nf), $m = 1, n = 1$ (**Ru-nf**); L: ciprofloxacin (cp), $m = 2, n = 2$ (**Ru-cp**); L: enrofloxacin (enro), $m = 0.5, n = 1$ (**Ru-enro**); L: ofloxacin (of), $m = 1, n = 1$ (**Ru-of**); L: levofloxacin (levof), $m = 2, n = 8$ (**Ru-levof**); DMSO: dimethylsulfoxide). In this regard we investigated, *in vitro*, the interaction of these complexes with double stranded calf thymus DNA through competitive binding studies with ethidium bromide, using fluorescence assays. For all complexes, a quenching in fluorescence of DNA-ethidium bromide complex was observed, suggesting a possible DNA intercalative binding.

Rezumat

Ca urmare a succesului reprezentat de cisplatin, câteva complexe metalice, altele decât platina au fost considerate de-a lungul anilor ca posibile alternative la cisplatină; în special, s-a constatat că unii compuși de ruteniu (III) posedă activități antitumorale și antimetastatice. Studiile din literatura de specialitate au relevat afinitatea acestor complecși pentru anumite biomolecule, cum ar fi ADN-ul și furnizează dovezi experimentale pentru formarea aducțiilor stabili cu acestea. Lucrarea de față prezintă proprietățile de legare la ADN ai unor complecși ai ruteniului (III) cu chinolone și dimetilsulfoxid(DMSO), cu formula generală $RuCl_3L_2(DMSO)_m \cdot nH_2O$ (L: acid pipemidic (pip), $m = 1, n = 2$ (**Ru-pip**); L: enoxacina (enx), $m = 1, n = 0$ (**Ru-enx**); L: norfloxacină (nf), $m = 1, n = 1$ (**Ru-nf**); L: ciprofloxacină (cp), $m = 2, n = 2$ (**Ru-cp**); L: enrofloxacină (enro), $m = 0,5, n = 1$ (**Ru-enro**); L: ofloxacină (of), $m = 1, n = 1$ (**Ru-of**); L: levofloxacină (levof), $m = 2, n = 8$ (**Ru-levof**), DMSO: dimetilsulfoxid). A fost studiată, *in vitro*, interacțiunea acestor complecși cu ADN-ul dublu catenar din timus de vițel prin studii de legare competitivă cu bromura de etidiu, folosind tehnici de fluorescență. Pentru toți complecșii a fost observată o stingere a semnalului fluorescent a complexului bromură de etidiu-ADN, ceea ce sugerează o posibilă intercalare a complecșilor la nivelul moleculei de ADN.

Keywords: ruthenium (III) complexes, quinolones, competitive binding studies, spectrofluorimetry

Introduction

Cancer is the second major cause of death after cardiovascular diseases [1-3]. Therefore developing new antitumour therapies represents a target for the pharmaceutical research worldwide. Many biomolecular experiments demonstrated that DNA is the primary intracellular target of anticancer drugs [4-7]. The design of new molecules that act on specific sites along a DNA helix has become a subject of considerable interest, because they may

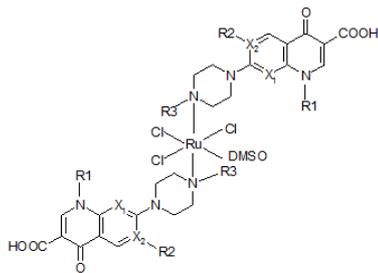
cause serious damage in tumour cells by blocking their division and resulting in neoplastic cell death. A more complete understanding of the targeted sites will lead to novel chemotherapeutic agents and also to developing highly sensitive diagnostic agents. Following the success of cisplatin, several metal complexes other than platinum have been considered over the years as possible alternatives to cisplatin, particularly it was found that ruthenium (III)

compounds possess antitumour and antimetastatic activities [7-9]. The literature studies revealed the affinity of these complexes for crucial biomolecules like DNA and provide evidence for the formation of stable adducts therewith [10-12].

On the other hand there are many reports in literature about the functional targets of quinolones and their interactions with DNA [13, 14]. They act as antibacterial drugs and are commonly used as treatment for many infections. They block DNA replication through the inhibition of type II and IV topoisomerases [15, 16].

The study of the biological and pharmacological properties of quinolones has been focused on their antibacterial activity [17-21], the interaction with DNA [22-25] and also the potential antitumour activity [26-28].

This paper presents the DNA-binding properties of some novel ruthenium (III) complexes with mixed ligands of the type $RuCl_3L_2(DMSO)_m \cdot nH_2O$ (L: pipemidic acid (pip), $m = 1, n = 2$ (**Ru-pip**); L: enoxacin (enx), $m = 1, n = 0$ (**Ru-enx**); L: norfloxacin (nf), $m = 1, n = 1$ (**Ru-nf**); L: ciprofloxacin (cp), $m = 2, n = 2$ (**Ru-cp**); L: enrofloxacin (enro), $m = 0.5, n = 1$ (**Ru-enro**); L: ofloxacin (of), $m = 1, n = 1$ (**Ru-of**); L: levofloxacin (levof), $m = 2, n = 8$ (**Ru-levof**); DMSO: dimethylsulfoxide), previously synthesized and characterized [29, 30]. All complexes display an octahedral stereochemistry with the quinolone ligand acting as monodentate coordinated through N4 atom of piperazinyl ring (Fig. 1).



Complex	X1	X2	R1	R2	R3
$[RuCl_3(pip)_2(DMSO)_2] \cdot 2H_2O$ (pip = pipemidic acid)	N	N	C_2H_5	-	H
$[RuCl_3(enx)_2(DMSO)]$ (enx = enoxacin)	N	C	C_2H_5	F	H
$[RuCl_3(nf)_2(DMSO)] \cdot H_2O$ (nf = norfloxacin)	CH	C	C_2H_5	F	H
$[RuCl_3(cp)_2(DMSO)] \cdot DMSO \cdot 2H_2O$ (cp = ciprofloxacin)	CH	C	Δ	F	H
$[RuCl_3(enro)_2(DMSO)] \cdot H_2O$ (enro = enrofloxacin)	CH	C	Δ	F	C_2H_5
$[RuCl_3(of)_2(DMSO)] \cdot H_2O$ (of = ofloxacin)	CH	C	Δ	F	CH_3
$[RuCl_3(levof)_2(DMSO)] \cdot DMSO \cdot 8H_2O$ (levof = levofloxacin)	CH	C	Δ	F	CH_3

Figure 1. Structures of the investigated Ru (III) complexes with quinolones

Ethidium bromide (3,8-diamino-5-ethyl -6-phenyl phenanthridium bromide, EtBr) is a planar cationic dye that strongly interacts with the DNA double

helix, by insertion of the phenanthridium ring between adjacent base pairs [31]. As a result of intercalation between DNA base pairs the enhancement of fluorescence emission of EtBr occurs [32]. In order to examine the ability of Ru(III) complexes to displace EtBr from intercalated complexes between EtBr and DNA, a competitive study was performed. This fluorescence-based competition can bring indirect evidence for the DNA-binding mode.

Materials and Methods

Materials

Type I collagen of bovine origin was extracted by the currently

During the experiment the following reagents were used: tris(hydroxymethyl)aminomethane hydrochloride, Tris-HCl (Merck), ethidium bromide, EtBr (Sigma Aldrich), calf thymus DNA (Sigma Aldrich), working solutions of the studied ruthenium (III) complexes.

DNA binding studies

All experiments were performed in Tris-HCl buffer, containing 5 mM Tris-HCl and 50 mM NaCl, adjusted to pH 7.4. The concentration of the calf thymus DNA (CT-DNA) solution was determined by measuring the absorption intensity at 260 nm, using the molar extinction coefficient value of $6600 M^{-1}cm^{-1}$. The absorbances registered at 260 nm and 280 nm gave the ratios of 1.84, indicating that the CT-DNA solution was sufficiently free of protein.

Fluorescence spectra were recorded on a Jasco FP 6500 spectrofluorometer at room temperature. A sample containing CT-DNA (10 μM) and EtBr (2 μM) was titrated with concentrated solutions containing the tested compounds. For every addition, the mixture was shaken and kept for 10 min at room temperature, and then the fluorescence emission spectra were recorded. Fluorescence emission spectra were recorded with excitation at 520 nm in the range 540 - 750 nm.

Results and Discussion

EtBr shows an intense fluorescence in the presence of CT-DNA, after the formation of the EtBr-DNA complex. Therefore, an EtBr-bound CT-DNA solution (2 μM EtBr + 10 μM DNA) has been used as a spectral probe. When the metal complexes bind to DNA, a decrease in the emission intensity of the EtBr-DNA complex is recorded (a quenching effect). The variation of the fluorescence emission intensity gives some information about the DNA binding affinity of the metal complexes and usually indicates the existence of stacking interactions (intercalation) between the DNA base pairs.

Data from the competitive binding studies have been plotted according to the classical Stern-Volmer equation [33]:

$$I_0/I = 1 + K_{sv} \cdot [Q],$$

where I_0 and I represent the fluorescence intensities in the absence and presence of the compound respectively, and $[Q]$ is the concentration of the tested compound (quencher).

The K_{sv} value is calculated as the slope of I_0/I versus $[Q]$ linear regression plot (Table I). The emission spectra of EtBr-DNA complex in the absence and presence of increasing concentrations of complexes are shown in Figures 1 - 7.

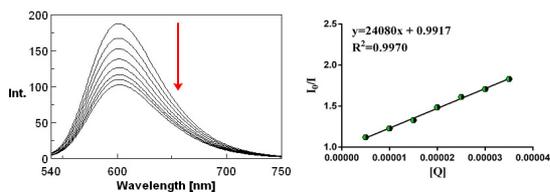


Figure 1.

Fluorescence spectra of the EtBr-DNA complex in the absence and in the presence of increasing amounts of complex **Ru-pip**: $\lambda_{ex} = 520$ nm, $\lambda_{em} = 601$ nm, $[EtBr] = 2$ μ M, $[DNA] = 10$ μ M, $[Ru-pip] = 5, 10, 15, 20, 25, 30, 35$ μ M.

Arrows indicate the changes in fluorescence intensities upon increasing the amounts of the tested compound (left). Plot of I_0/I vs. $[Q]$ for the system **Ru-pip**-EtBr-DNA (right).

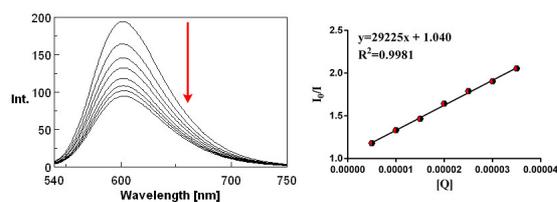


Figure 2.

Fluorescence spectra of the EtBr-DNA complex in the absence and in the presence of increasing amounts of complex **Ru-enx**: $\lambda_{ex} = 520$ nm, $\lambda_{em} = 601$ nm, $[EtBr] = 2$ μ M, $[DNA] = 10$ μ M, $[Ru-enx] = 5, 10, 15, 20, 25, 30, 35$ μ M.

Arrows indicate the changes in fluorescence intensities upon increasing the amounts of the tested compound (left). Plot of I_0/I vs. $[Q]$ for the system **Ru-enx**-EtBr-DNA (right).

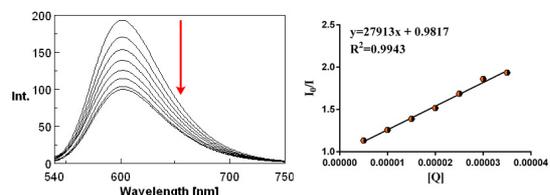


Figure 3.

Fluorescence spectra of the EtBr-DNA complex in the absence and in the presence of increasing amounts of complex **Ru-nf**: $\lambda_{ex} = 520$ nm, $\lambda_{em} = 601$ nm, $[EtBr] = 2$ μ M, $[DNA] = 10$ μ M, $[Ru-nf] = 5, 10, 15, 20, 25, 30, 35$ μ M.

Arrows indicate the changes in fluorescence intensities upon increasing the amounts of the tested compound (left). Plot of I_0/I vs. $[Q]$ for the system **Ru-nf**-EtBr-DNA (right).

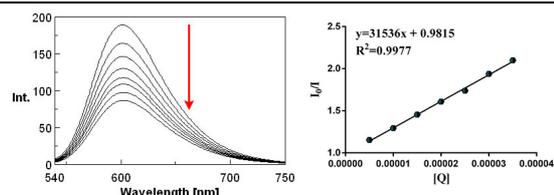


Figure 4.

Fluorescence spectra of the EtBr-DNA complex in the absence and in the presence of increasing amounts of complex **Ru-cp**: $\lambda_{ex} = 520$ nm, $\lambda_{em} = 601$ nm, $[EtBr] = 2$ μ M, $[DNA] = 10$ μ M, $[Ru-cp] = 5, 10, 15, 20, 25, 30, 35$ μ M. Arrows indicate the changes in fluorescence intensities upon increasing the amounts of the tested compound (left). Plot of I_0/I vs. $[Q]$ for the system **Ru-cp**-EtBr-DNA (right).

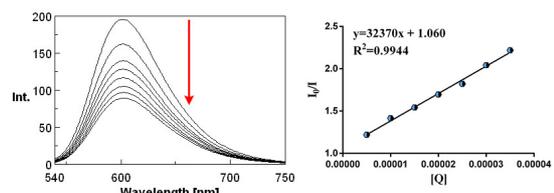


Figure 5.

Fluorescence spectra of the EtBr-DNA complex in the absence and in the presence of increasing amounts of complex **Ru-enro**: $\lambda_{ex} = 520$ nm, $\lambda_{em} = 601$ nm, $[EtBr] = 2$ μ M, $[DNA] = 10$ μ M, $[Ru-enro] = 5, 10, 15, 20, 25, 30, 35$ μ M. Arrows indicate the changes in fluorescence intensities upon increasing the amounts of the tested compound (left). Plot of I_0/I vs. $[Q]$ for the system **Ru-enro**-EtBr-DNA (right).

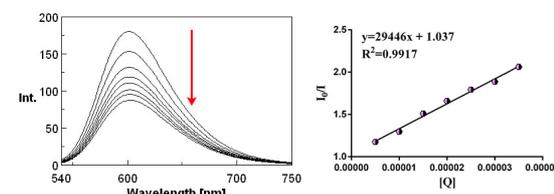


Figure 6.

Fluorescence spectra of the EtBr-DNA complex in the absence and in the presence of increasing amounts of complex **Ru-of**: $\lambda_{ex} = 520$ nm, $\lambda_{em} = 601$ nm, $[EtBr] = 2$ μ M, $[DNA] = 10$ μ M, $[Ru-of] = 5, 10, 15, 20, 25, 30, 35$ μ M. Arrows indicate the changes in fluorescence intensities upon increasing the amounts of the tested compound (left). Plot of I_0/I vs. $[Q]$ for the system **Ru-of**-EtBr-DNA (right).

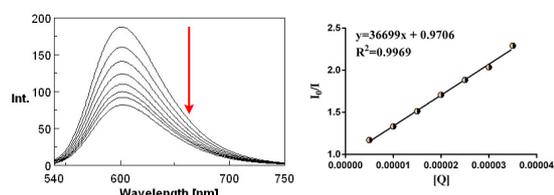


Figure 7.

Fluorescence spectra of the EtBr-DNA complex in the absence and in the presence of increasing amounts of complex **Ru-levof**: $\lambda_{ex} = 520$ nm, $\lambda_{em} = 601$ nm, $[EtBr] = 2$ μ M, $[DNA] = 10$ μ M, $[Ru-levof] = 5, 10, 15, 20, 25, 30, 35$ μ M. Arrows indicate the changes in fluorescence intensities upon increasing the amounts of the tested compound (left). Plot of I_0/I vs. $[Q]$ for the system **Ru-levof**-EtBr-DNA (right).

Table I
Values of linear Stern-Volmer constants obtained for the studied Ru(III) complexes

Compound	$K_{sv} (M^{-1})$
Ru-pip	2.41×10^4
Ru-enx	2.92×10^4
Ru-nf	2.79×10^4
Ru-cp	3.15×10^4
Ru-enro	3.24×10^4
Ru-of	2.94×10^4
Ru-levof	3.67×10^4

The fluorescence intensity of DNA-EtBr system decreased upon increasing the concentration of complexes without major changes in the wavelength of maximum emission. The quenching of EtBr bound to DNA is in good agreement with the linear Stern-Volmer equation:

$$I_0/I = 1 + K_{sv} \cdot [Q].$$

The K_{sv} values are $\sim 10^4 M^{-1}$ for all complexes suggesting that they can insert between DNA base pairs. In regard to the spectral general features, a strong resemblance of the seven complexes can be noticed, therefore a similar binding mechanism to DNA is to be expected.

Conclusions

In order to evaluate the ability of novel Ru(III) complexes to interact with DNA, a competitive study was performed: the fluorescence intensity of DNA-EtBr system was registered in the absence and in the presence of increasing amounts of complexes. A quenching of the fluorescence intensity of DNA-EtBr system was observed for all complexes suggesting that they can interact with DNA through an intercalative mode.

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