10750 *Inorg. Chem.* **2010**, *49*, 10750–10752
DOI: 10.1021/ic101355d

Inorganic Chemistry
Communication

First Ruthenium Organometallic Complex of Antibacterial Agent Ofloxacin. Crystal Structure and Interactions with DNA

Iztok Turel,*,† Jakob Kljun,† Franc Perdih,† Elena Morozova,‡ Vladimir Bakulev,‡ Nina Kasyanenko,*,‡ Jo Ann W. Byl,§ and Neil Osheroff§

†*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva 5, 1000 Ljubljana, Slovenia,* ‡*Department of Molecular Biophysics, Faculty of Physics, St. Petersburg State University, Uluanovskaya St. 1, Petrodvorets, St. Petersburg 198504, Russia, and* §*Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, United States*

Received July 7, 2010

An organometallic ruthenium complex of quinolone antibacterial agent ofloxacin, [(η^6-*p*-cymene)RuCl(*O*,*O*-oflo)]·2.8H_2O (**1**·2.8H_2O), was isolated, and its crystal structure was determined. In this "piano-stool" complex, quinolone is bidentately coordinated to the metal through the ring carbonyl and one of the carboxylic oxygen atoms. Interactions of the title complex with DNA were studied by spectroscopic methods [electronic, fluorescence, and circular dichroism (CD)] and atomic force microscopy (AFM). It was established that the electrostatic attraction between the ruthenium complex and DNA in a solution is important for binding because interactions were observed only in a solution with low ionic strengths. An induced-CD (ICD) signal was observed in a solution of DNA and the title complex, which proves interaction between ruthenium and macromolecules. Competitive binding between cisplatin and **1** to DNA revealed that cisplatin prevents binding of **1**. Our experiments revealed that binding of the title complex to DNA occurs also if guanine N7 is protonated. AFM has shown that the title complex provokes DNA shrinkage. Preliminary biological tests have also been performed.

Ruthenium anticancer complexes have been extensively studied and two of them, NAMI-A and KP1019, respectively have successfully entered clinical trials.[1] Organometallic ruthenium complexes are also potential anticancer agents that show promising activity.[2]

Ofloxacin (ofloH; Scheme 1) belongs to a group of quinolone antibacterial agents and is successfully used in clinical practice.[3] The target of the quinolone is the enzyme DNA gyrase, and it is also well established that quinolone interacts with calf thymus DNA.[4] The mechanism of action of these drugs is not fully understood, but several authors have stressed the importance of magnesium ions in these interactions.[5] It is well-known that metal ions coordinate to quinolone and some complexes exert biological activity.[6] The synthesis and study of metal complexes with drugs used in clinical practice, which may exhibit synergistic activity, has attracted much attention as an approach to new drug development.[7] The crystal structures of ofloxacin complexes with copper, zinc, cobalt, and magnesium were reported before.[6b,8] We have prepared and characterized the first ruthenium organometallic complex of ofloxacin, [(η^6-*p*-cymene)RuCl(*O*,*O*-oflo)]·2.8H_2O (**1**·2.8H_2O). Because it is known that both ruthenium and ofloxacin interact with DNA, it was also appealing to test how the title complex interacts with DNA.

The title complex **1** can be prepared by treating the ligand with NaOH and ruthenium precursor ([RuCl(μ-Cl)(η^6-*p*-cymene)]$_2$, **P1**) in methanol. The microcrystallinic product was dissolved in CH_2Cl_2, and orange-brown crystals of **1**·2.8H_2O suitable for X-ray analysis were obtained by slow evaporation of a solvent. The complex adopts the pseudooctahedral "piano-stool" geometry, with ruthenium(II) π-bonded to the *p*-cymene ring and σ-bonded to a chloride and two oxygen atoms of the chelated quinolone ligand (Figure 1 and Table S1 in the Supporting Information, SI).

*To whom correspondence should be addressed. E-mail: iztok.turel@fkkt.uni-lj.si (I.T.), nkasyanenko@mail.ru (N.K.).

(1) (a) Rademaker-Lakhai, J. M.; van den Bongard, D.; Pluim, D.; Beijnen, J. H.; Schellens, J. H. M. *Clin. Cancer Res.* **2004**, *10*, 3717–3727. (b) Hartinger, C. G.; Zorbas-Seifried, S.; Jakupec, M. A.; Kynast, B.; Zorbas, H.; Keppler, B. K. *J. Inorg. Biochem.* **2006**, *100*, 891–904. (c) Sava, G.; Alessio, E.; Bergamo, A.; Mestroni, G. In *Topics in Biological Inorganic Chemistry*; Clarke, M. J., Sadler, P. J., Eds.; Springer Verlag: Berlin, 1999; pp 143−169.
(2) Hartinger, C. G.; Dyson, P. J. *Chem. Soc. Rev.* **2009**, *38*(2), 391–401.
(3) Brighty, K. E.; Gootz, T. D.; Andriole, V. T. *The Quinolones*; Academic Press: San Diego, 2000.
(4) Morrissey, I.; Hoshino, K.; Sato, K.; Yoshida, A.; Hayakawa, I.; Bures, M. G.; Shen, L. L. *Antimicrob. Agents Chemother.* **1996**, *40*, 1775–1784.
(5) Mitscher, L. A. *Chem. Rev.* **2005**, *105*, 559–592.
(6) (a) Turel, I. *Coord. Chem. Rev.* **2002**, *232*, 27–47. (b) Macias, B.; Villa, M. V.; Rubio, I.; Castineiras, A.; Borras, J. *J. Inorg. Biochem.* **2001**, *84*, 163–170. (c) Katsarou, M. E.; Efthimiadou, E. K.; Psomas, G.; Karaliota, A.; Vourloumis, D. *J. Med. Chem.* **2008**, *51*, 470–478.
(7) Christofis, P.; Katsarou, M.; Papakyriakou, A.; Sanakis, Y.; Katsaros, N.; Psomas, G. *J. Inorg. Biochem.* **2005**, *99*, 2197–2210.
(8) (a) Drevensek, P.; Kosmrlj, J.; Giester, G.; Skauge, T.; Sletten, E.; Sepcic, K.; Turel, I. *J. Inorg. Biochem.* **2006**, *100*, 1755–1763. (b) Yu, L. C.; Lai, L.; Xia, R.; Liu, S. L. *J. Coord. Chem.* **2009**, *62*, 1313–1319.

 Published on Web 10/26/2010

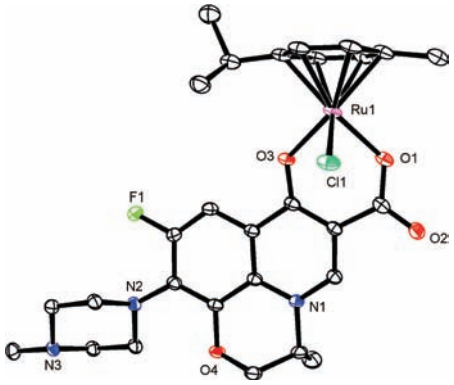
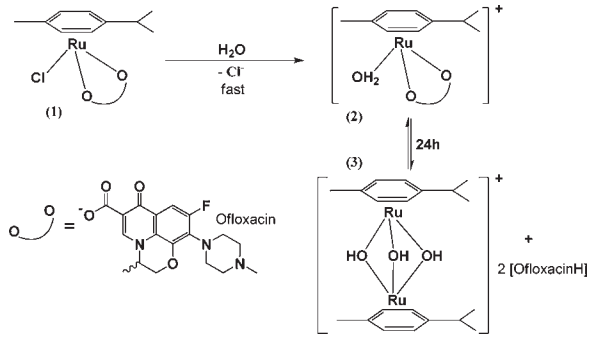


Figure 1. ORTEP diagram of $\mathbf{1}\cdot 2.8H_2O$ with thermal ellipsoids drawn at the 30% probability level. Hydrogen atoms are omitted for clarity. Selected bond lengths (Å) and angles (deg): Ru1−Cl1 2.4183(7), Ru1−O1 2.069(2), Ru1−O3 2.0713(18), O1−C6 1.293(3), O3−C9 1.275(3); O1−Ru1−O3 85.30(7), O1−Ru1−Cl1 83.73(6), O3−Ru1−Cl1 86.92(6).

Scheme 1. Main Products of **1** Hydrolysis: **2** and Dimer **3**



In contrast to most metal quinolone complexes reported so far, which are only sparingly soluble,[6a] the title complex is easily soluble in water (solubility 0.016 M at room temperature). We have realized that, after dissolution, hydrolysis occurs, which was already reported for other ruthenium organometallic complexes with various ligands.[9] We have studied these processes by NMR spectroscopy and electrospray ionization mass spectrometry (ESI-MS), and the results are similar to those obtained by others.[9] It can be concluded that first quick hydrolysis of the chloride ion is taking place (product **2**), which is followed by the formation of a dimer[9] (Scheme 1 and Figures S1−S3 and Table S2 in the SI). However, we can clearly see from NMR experiments (Figure S1 in the SI) that even after 1 day a substantial amount of the first hydrolysis product (58%) is still present in solution.

It was established that interaction of the title complex **1** with DNA is observed only in a solution with low ionic strengths. This is clear evidence that the electrostatic attraction between the ruthenium complex and DNA in a solution is important for binding. We assume that negatively charged DNA interacts with the positively charged hydrolytic products of **1**. Fluorescence data of solutions containing DNA and **1** (Figure S4 in the SI) indicate two types of binding. The first type of binding coincides with the ofloxacin binding

(9) (a) Peacock, A. F. A.; Melchart, M.; Deeth, R. J.; Habtemariam, A.; Parsons, S.; Sadler, P. J. *Chem.—Eur. J.* **2007**, *13*, 2601–2613. (b) Grguric-Sipka, S.; Stepanenko, I. N.; Lazic, J. M.; Bartel, C.; Jakupec, M. A.; Arion, V. B.; Keppler, B. K. *Dalton Trans.* **2009**, *17*, 3334–3339.

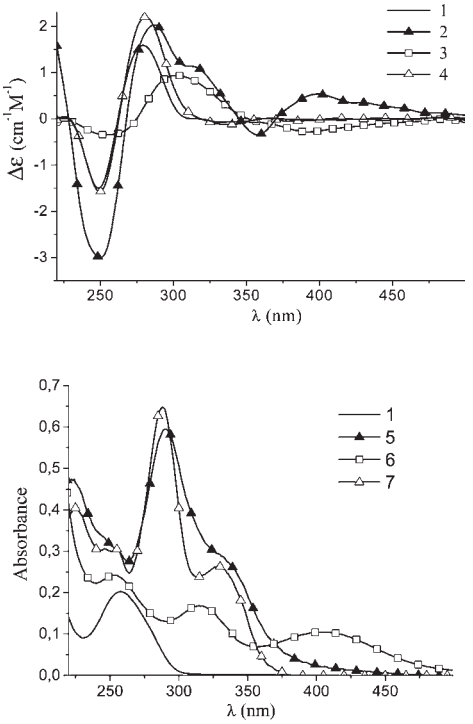


Figure 2. CD (above) and UV−vis (below) spectra of free DNA (1), **1** (5), precursor **P1** (6), ofloH (7), and DNA complexes with **1** (2), precursor **P1** (3), and ofloH (4) in 5 mM NaCl. $C(\mathbf{1}) = 2.1 \times 10^{-5}$ M, $C(\mathbf{P1}) = 8 \times 10^{-5}$ M, C(ofloH) $= 2.2 \times 10^{-5}$ M, C(DNA) $= 7.5 \times 10^{-5}$ (CD) and 1.5×10^{-5} (UV−vis) M (bp).

mode with binding constant $K = 1.2 \times 10^6$ and a maximal number of bound ruthenium atoms per 1 DNA base pair $n = 0.02$ (K and n were determined from the curve equation). The second type of binding is well recognized at $r > 0.02$ [r is the ratio of total concentrations of fluorophores (kept constant in the experiment) and DNA base pairs in a solution; Figure S4 in the SI] and corresponds to the ruthenium complex binding with DNA. The latter type of binding was analyzed also with UV−vis spectroscopy and circular dichroism (CD) titration experiments (see below; Figure 2). As we can conclude from the hydrolysis study (mentioned above), the main species in an aqueous (as well as in 5 mM NaCl) solution of **1** are ruthenium containing hydrolytic products and free ofloxacin. Each of these can interact with DNA differently. We have established that the CD spectrum obtained by mixing solutions of DNA and **1** (Figure 2) is not just a simple mathematical sum of the DNA−ofloxacin and DNA−**P1** spectra. The title complex does not have signals in the CD spectrum. However, when DNA was added to the title complex solution, an induced-CD (ICD) spectrum was obtained (the signal is out of DNA and ofloH absorption bands). ICD at $\lambda > 380$ nm corresponds to the interaction of ruthenium with DNA because from all reactants used only the precursor **P1** has an absorption band at around 400 nm. However, the shape of the CD spectrum for the DNA−**P1** solution is substantially different in this region. From these facts, we can propose that, in the DNA complex with the hydrolytic products of **1**, ruthenium is bound to the quinolone.

Analysis of the ICD spectrum at constant **1** and different DNA concentrations (Figure S5 in the SI) indicates the number of binding sites $n = 0.3$ for **1**. This result was obtained from the saturation of **1** binding with DNA from the ICD

dependence on *r*. It is essential to note that this analysis takes into account only DNA interaction with intact **2**, which is accompanied with ICD and does not reflect other binding modes.

To get more details on the type of interaction between the title complex and DNA, competitive binding experiments with cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, were performed. From these experiments, it is clear that cisplatin and **1** compete for binding positions on DNA (Figure S6 in the SI). Complex **1** (or cisplatin) was added into the initial solution of DNA, and after 1 day, the second compound, cisplatin (or **1**), was added. The concentration of cisplatin was 5×10^{-5} M because at this concentration almost all of the binding sites on DNA are occupied but its double-stranded structure is still stable. For all systems under the study, the spectra were recorded on the third day. The CD spectra show that **1** could not bind to DNA after cisplatin had already coordinated (no typical ICD band appeared). On the other hand, ICD bands (negative band at 345 nm and positive band at 400 nm) in solution of **1** and DNA do not disappear after the addition of cisplatin. This is proof that also **1** strongly interacts with DNA and cisplatin cannot simply replace it. One possibility would be that there is a competition between cisplatin and **1** for the guanine N7 atom of DNA. However, our experiments at different pH values revealed that binding of the title complex occurs also when N7 is protonated (Figure S7 in the SI). This is proof that ruthenium binding to N7 does not occur (or at least is not crucial). An alternative interpretation is that binding of cisplatin (or **1**) causes modification of the DNA secondary structure and, as a result, the steric inconvenience prevents further binding of the second coordination compound to DNA. It is known that also cisplatin is prone to hydrolysis (one or both chlorides are displaced by aqua ligands). In the cell, the resulting cationic species react with DNA to give numerous cisplatin−DNA adducts.[10] In the most important adduct, cisplatin is chelated to two neighboring guanines at their N7 sites to form intrastrand cross-links, resulting in a kink of the DNA structure.[11] We can suppose, for example, that such Pt−N7 guanine binding and the corresponding change of the DNA geometry prevent ruthenium binding to DNA.

At the moment, the details of the title complex−DNA binding are not known, but some assumptions can be done from our experimental results and the available literature data on similar systems (however, we should be aware that the systems are not ideally comparable). It is interesting to note a recently published structure of a topoisomerase enzyme−DNA−moxifloxacin quinolone complex.[12] It was revealed that the role of metal (Mg^{2+}) is very important. Magnesium is bidentately coordinated by the quinolone and four aqua ligands and mediates interactions with the DNA. There is no direct coordination between metal and DNA, but coordinated water molecules are involved in hydrogen bonding with DNA nucleobases. Sadler et al. have studied the binding of various $[(\eta^6\text{-arene})Ru(en)]^{2+}$ complexes to nucleotide phosphate groups. It was found that ruthenium interacts with phosphate, though it was suggested that in DNA the direct coordination of ruthenium to backbone phosphodiester groups is probably weak. However, electrostatic interactions and hydrogen bonding may be involved in the initial recognition of the ruthenium complex prior to binding to guanine N7, which is similar to some platinum complexes.[13] We can therefore propose that also the positively charged hydrolytic products of **1** are first attracted to negatively charged DNA. After that, binding of ruthenium to DNA occurs. Because our experiments revealed that binding to guanine N7 atoms is not crucial, other types of interaction (interaction with phosphate groups, hydrogen bonds, etc.) might be more important. It is also important to stress that our AFM experiments (Figure S8 in the SI) have shown that the title complex caused DNA shrinkage. At a high concentration of **1**, the formation of rather uniform compressed structures was observed but not condensation of DNA, which was, for example, recently observed in ruthenium complexes that can intercalate into DNA.[14] Compound **1** was tested in in vitro tests against various microorganisms that are causing tropical diseases and in in vitro cytotoxicity experiments with rat skeletal myoblasts. The results have shown that compound **1** is moderately active against *Trypanosoma b. rhodesiense*, *Trypanosoma cruzi*, and *Plasmodium falciparum*, while precursors **P1** and ofloH are moderately active only against *P. falciparum* (Table S3 in the SI). The enzyme inhibition tests (human topoisomerase IIα) have also been performed. Compound **1** shows no improved activity in comparison to free ofloH (Figure S9 in the SI).

Acknowledgment. We are thankful for financial support from the Slovenian Research Agency (J1-0200-0103-008; EN → FIST Centre of Excellence, Dunajska 156, SI-1000 Ljubljana, Slovenia) and the Ministry of Education and Science of Russian Federation and the Federal Agency of Education (Grant AVTP "Development of Science Potential of High School" 4011). We thank Dr. B. Kralj and Dr. D. Žigon (Jožef Stefan Institute, Ljubljana, Slovenia) for MS measurements. We thank Dr. J. Keiser and M. Kaiser (Swiss Tropical and Public Health Institute, Basel, Switzerland) for tropical disease tests.

Supporting Information Available: X-ray crystallographic data in CIF format, experimental details, characterization data of compound **1**, X-ray diffraction data, ESI-MS, NMR, fluorescence, CD, AFM, biological tests, Figures S1−S9, Tables S1−S3, and additional literature data. This material is available free of charge via the Internet at http://pubs.acs.org.

(10) Reedijk, J. *Eur. J. Inorg. Chem.* **2009**, *10*, 1303–1312.
(11) Jung, Y. W.; Lippard, S. J. *Chem. Rev.* **2007**, *107*, 1387–1407.
(12) Wohlkonig, A.; Chan, P. F.; Fosberry, A. P.; Homes, P.; Huang, J.; Kranz, M.; Leydon, V. R.; Miles, T. J.; Pearson, N. D.; Perera, R. L.; Shillings, A. J.; Gwynn, M. N.; Bax, B. D. *Nat. Struct. Mol. Biol.* **2010**, *17*, 1152–1153.
(13) Chen, H. M.; Parkinson, J. A.; Morris, R. E.; Sadler, P. J. *J. Am. Chem. Soc.* **2003**, *125*, 173–186.
(14) Sun, B.; Guan, J. X.; Xu, L.; Yu, B. L.; Jiang, L.; Kou, J. F.; Wang, L.; Ding, X. D.; Chao, H.; Ji, L. N. *Inorg. Chem.* **2009**, *48*, 4637–4639.