Inorg. Chem. **2005**, *44*, 4897–4899

Structure–Function Relationships within Keppler-Type Antitumor Ruthenium(III) Complexes: the Case of 2-Aminothiazolium[*trans*-tetrachlorobis(2-aminothiazole)ruthenate(III)]

Pasquale Mura,*,† Francesca Piccioli,‡ Chiara Gabbiani,§ Mercedes Camalli,† and Luigi Messori*,‡

Institute of Crystallography sezione di Monterotondo, Area della Ricerca di Roma, CNR, CP10, I-00016 Monterotondo Stazione, Rome, Italy, Department of Chemistry, University of Florence, Via della Lastruccia 3, I-50019 Sesto Fiorentino (FI), Italy, and CIRCMSB, Local Unit of Florence, University of Florence, Florence, Italy

Received February 4, 2005

Keppler-type ruthenium(III) complexes exhibit promising antitumor properties. We report here a study of 2-aminothiazolium[*trans*-tetrachlorobis(2-aminothiazole)ruthenate(III)], both in the solid state and in solution. The crystal structure has been solved and found to exhibit classical features. Important solvatochromic effects were revealed. Notably, we observed that introduction of an amino group in position 2 greatly accelerates chloride hydrolysis compared to the thiazole analogue; this latter finding may be of interest for a fine-tuning of the reactivity of these novel metallodrugs.

Ruthenium(III) compounds represent a new family of promising metal-based anticancer drugs;[1] presently, some of them are being tested as drug candidates in the clinic. For instance, NAMI-A, a sulfoxide ruthenium(III) complex developed in Trieste, endowed with outstanding antimetastatic properties, has successfully completed phase I clinical trials;[2] similarly, KP1019, a representative of the so-called "Keppler-type" complexes, of general formula $L^+(L_2RuCl_4)^-$, showed an encouraging preclinical profile and is now undergoing phase I studies.[3]

Despite the numerous in vitro and preclinical investigations, the mechanism of action of antitumor ruthenium(III) complexes is still poorly understood. A number of studies point out that the interactions of these ruthenium(III) complexes with DNA are weaker than in the case of related platinum(II) complexes, suggesting the existence of different biomolecular targets.[4] Thus, the paradigm of cisplatin mechanism, i.e., "direct DNA damage → cytotoxic effect → cell death through apoptosis", barely applies to ruthenium(III) complexes whose cytotoxic effects are generally rather modest. On the other hand, significant interactions of anticancer ruthenium(III) compounds with potential protein targets were recently described.[5]

These arguments led us to perform detailed chemical and pharmacological investigations on a few Keppler-type ruthenium(III) complexes to reveal possible structure−function relationships. In particular, synthetic strategies are being devised to modulate finely the reactivity of the ruthenium(III) center with respect to the redox properties and to the kinetics of ligand exchange. We report here on the main chemical features of a Keppler-type compound where L is 2-aminothiazole; results are compared to those of the recently described thiazole analogue.[6]

The complex 2-aminothiazolium[*trans*-tetrachlorobis(2-aminothiazole)ruthenate(III)], (**I**), for which previous studies had already evidenced some significant in vivo antitumor activity against P388 leukemia,[7] was prepared according to standard synthetic procedures.[8] The crystal structure of **I**, solved in our laboratory, is shown in Figure 1.

The complex essentially manifests a classical octahedral geometry around the ruthenium(III) center. The 2-aminothiazole ligands are axially bound to the metal through the heteroaromatic nitrogen atom. It is of interest to note that 2-aminothiazole behaves here as a monodentate ligand despite the fact that it is potentially bidentate; this is probably due to the soft reaction conditions of the synthetic procedure.[8] More-drastic reaction conditions (i.e., reflux reaction after

* To whom correspondence should be addressed. E-mail: Pasquale.Mura@ic.cnr.it (P.M.); luigi.messori@unifi.it (L.M.). Fax: +390690672630 (P.M.); +390554573385 (L.M.). Tel: +390690672615 (P.M.); +390554573284 (L.M.).

† Institute of Crystallography.

‡ Department of Chemistry, University of Florence.

§ CIRCMSB, Local Unit of Florence, University of Florence.

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10.1021/ic050194p CCC: $30.25
Published on Web 06/15/2005

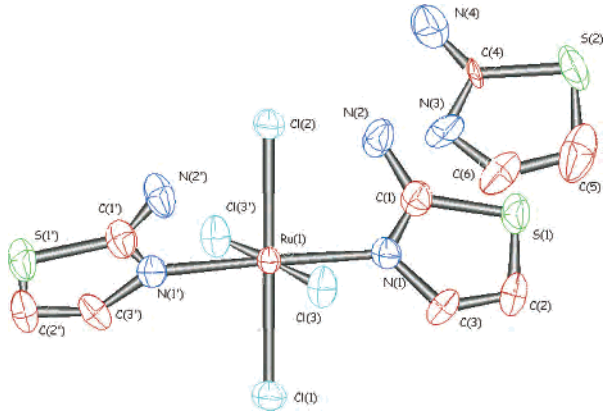

Figure 1. Geometry[13] of compound **I**. The Ru, Cl(1), and Cl(2) atoms lie on a 2-fold rotation axis. Thermal ellipsoids are drown at the 50% probability level. Selected bond distances (Å): Ru(1)−Cl(1) = 2.350(3), Ru(1)−Cl(2) = 2.360(3), Ru−Cl(3) = 2.365(2), Ru−N(1) = 2.138(9).

ligand addition) may give rise to a small chelate ring complex, as proposed by Nikolova et al.[10] Compound **I**[11] is isostructural with ICR;[9] the asymmetric unit contains half of the complex with the Ru, Cl(1), and Cl(2) atoms lying on a 2-fold rotation axis; half of the 2-aminothiazolium cation is disordered around a crystallographic inversion center with the atoms having an occupancy factor of 0.5. The Ru−Cl distances in **I** (mean value Ru−Cl = 2.358(3) Å), in ICR[9] (mean value Ru−Cl = 2.349(1) Å) and in TzICR (mean value Ru−Cl = 2.352(3) Å) are almost equal, considering the standard deviations; the same holds for the Ru−N distance, 2.138(9) Å in **I**, 2.079(3) Å in ICR, and 2.083(8) Å (mean value) in TzICR, considering the standard deviations.[6,9] The planes of the two coordinated *trans*-2-aminothiazole ligands in **I** are nearly perpendicular to one another (91.7°) like in ICR (84°).[9] Remarkably, the nitrogens of the amino groups are very close to the adjacent chlorine atom—the N(2)−Cl(2) and N(2′)−Cl(3′) distances are both 3.189(9) Å—suggesting the existence of intramolecular hydrogen bonds.

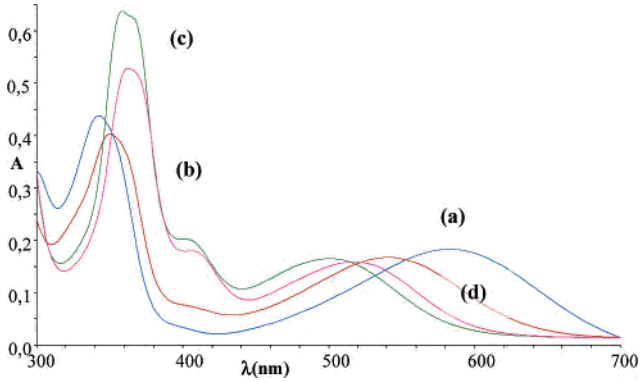

Figure 2. Solvatochromism of the complex: the absorption spectra in aqueous solution (a), DMSO (b), acetonitrile (c), and EtOH (d) are reported. The concentration of **I** is 1×10^{-4} M.

After determining the main structural features of compound **I**, we focused our attention on its solution behavior. Freshly prepared aqueous solutions of **I** are characterized by a few intense bands in the visible spectrum, which are CT in nature (located at 300, 344, and 580 nm, respectively), plus an intense band at 242 nm. Notably all these bands, but in particular the one at low energy, are very sensitive to the nature of the solvent, providing a clear example of solvatochromism.[15] For instance, the band located at 580 nm (in aqueous solutions) moves to 520 nm when the compound is dissolved in DMSO and to 500 nm in acetonitrile. Representative spectra of compound **I** in different solvents are shown in Figure 2.

Complex **I** is stable for several days when dissolved in organic solvents e.g., DMSO, ethanol, acetonitrile, etc., but exhibits large time-dependent spectral changes in aqueous media. Indeed, Keppler-type compounds are known to undergo progressive hydrolysis of their chloride groups that is reflected into characteristic spectral modifications.[16] Chloride hydrolysis is generally slow and takes several hours, or even days, to reach completion at room temperature. In some cases, the hydrolysis processes of Keppler-type compounds have been described in great detail and the main species deriving from hydrolysis unambiguously identified.[16,17]

The time dependence of the absorption spectra of compound **I** dissolved in the phosphate buffer (NaH_2PO_4 50mM, NaCl 100mM, pH 7.4) is shown in Figure 3. The progressive decrease of the bands at 344 and 580 nm is noted. The

(8) $RuCl_3 \cdot H_2O$ (0.21 g, 1 mmol) was dissolved in 6 mL of ethanol and 6 mL of 1 N HCl and gently refluxed for 2 h. After the mother liquor was cooled at room temperature, 0.60 g (6 mmol) of 2-aminothiazole, dissolved in 2 mL of ethanol and 1 mL of 6 N HCl, was added while stirring. The mixture was stirred for 30 min at room temperature; then, the mother liquor was left for 6 days at room temperature. An appreciable quantity of product formed. The deep-violet crystals were filtered off, washed with ethanol and ether, and dried in a vacuum. Yield: 0.32 g (59%). Anal. Calcd for $C_9H_{13}Cl_4N_6RuS_3$ (M = 544.32): C, 19.86; H, 2.41; N, 15.44; S, 17.67%. Found: C, 19.70; H, 2.57; N, 15.16; S, 17.26%. The infrared spectra of **I** show two bands at 328.0 and 284.0 cm^{-1} attributed to Ru−Cl stretching vibrations and a band at 250.0 cm^{-1} that may be attributed to ν_{Ru-N}.[6, 9]

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(11) $C_9H_{13}Cl_4N_6RuS_3$, M = 544.32, monoclinic, space group $C2/c$, a = 12.928(1) Å, b = 8.350(1) Å, c = 18.453(2) Å, β = 115.40(1)°, V = 1799.4(4) Å^3, Z = 4, D_c = 2.010, μ = 15.964 mm^{-1} (Cu Kα, λ = 1.54178 Å), room temperature. Total no. of data 1416 (2Θ range-(deg): 5−124), R value for equiv. reflns 0.042; no. of observed data 1279 ($I_o > 3\sigma(I_o)$), final residuals (for 1279 data, after introduction of the fixed contributions of the H atoms, apart those of the disordered 2-aminothiazolium) are R = 0.048 and R_w = 0.069, GOF = 0.91. Data collection was performed using a RigakuAFC5 diffractometer; no decay correction was applied. Data were corrected for Lorenz and polarization effects. An empirical absorption correction, based on azimuthal scans of several reflections, was applied to intensities.[12] The structure was solved using SIR97 program[13] and refined as full matrix in the least-squares calculation using CAOS programs.[14]

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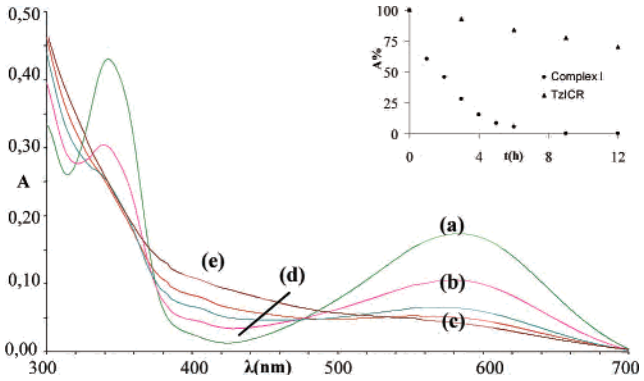

Figure 3. Hydrolysis profiles of **I** in phosphate buffer, the spectra were collected at $t = 0$ (a), 2 (b), 4 (c), 6 (d), and 12 h (e); the concentration of the complex is 1×10^{-4} M. The insert reports the percent variation of the main visible band of **I** compared to TzICR (from ref 6)

observation of two isosbestic points at 700 and 480 nm is indicative of an equilibrium between the starting species and the first hydrolyzed one (the monoaqua complex). These spectral changes are complete within ca. 6 h; remarkably, the spectrum recorded after 12 h (trace e) provides evidence of the emergence of a second process. At variance, hydrolysis of *trans*-tetrachlorobis(thiazole)ruthenate(III)] in the same phosphate buffer is characterized by a much slower decrease in intensity of the band at 360 nm; after 24 h, less than 20% of the *trans*-tetrachlorobis(thiazole)ruthenate(III)] anion has undergone hydrolysis of the first chloride group.[6] A direct comparison of the rates of the first hydrolytic process for the two compounds is provided in Figure 3, insert.

Thus, the data reported here point out that replacement of thiazole with the more-basic 2-aminothiazole produces a large increase of the rate of the first chloride hydrolysis. This result is in line with previous observations on promotion of chloride hydrolysis by an increase of the basicity of the nitrogen heterocyclic ligand.[18,19] In other words, dechlorination of these ruthenium(III) complexes is favored by an increase of electron transfer from the nitrogen to the metal with a concomitant weakening of the metal−chloride bonds.

Pairwise, the increase in basicity of the coordinated nitrogen is believed to stabilize ruthenium in the +3 oxidation state.[18] In conclusion, the present results suggest that introduction of a amino substituent on the heterocyclic axial ligand, while not altering the general architecture of the ruthenium(III) complex, increases the basicity of the coordinated nitrogen and thus produces relevant effects on the electronic structure and the reactivity of the metal center. This observation may open the way to specific synthetic strategies for a fine-tuning of the kinetic properties of the ruthenium(III) center. Preliminary studies, recently performed in our laboratory, point out that the two mentioned complexes differ markedly in their interaction patterns with model proteins. We propose that the differences observed in the solution behavior of these two strictly related compounds may have a significant impact on their pharmacological activity.

Acknowledgment. Cassa di Risparmio di Firenze and MIUR PRIN 2004 are gratefully acknowleged for financial support.

Supporting Information Available: Crystallographic information in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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