Journal of Medicinal Chemistry

Volume 50, Number 24 November 29, 2007

Letters

Activity of Rat Cytosolic Thioredoxin Reductase Is Strongly Decreased by *trans*-[Bis(2-amino-5-methylthiazole)tetrachlororuthenate(III)]: First Report of Relevant Thioredoxin Reductase Inhibition for a Ruthenium Compound

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Received July 16, 2007

Abstract: A novel "Keppler type" ruthenium(III) compound *trans*-[bis(2-amino 5-methylthiazole)tetrachlororuthenate(III)] **1**, of potential interest as an anticancer agent, was designed, synthesized, and characterized. Its interactions with various proteins were analyzed, including the selenoenzyme thioredoxin reductase, an emerging target for anticancer metallodrugs. The selective inhibition of the cytosolic form of this selenoenzyme was documented, this being the first report of significant thioredoxin reductase inhibition by a ruthenium compound.

Mammalian thioredoxin reductases (TrxR[a]) are large homodimeric proteins that play a major role in the intracellular redox metabolism, together with a few other biochemical systems.[1,2] Thioredoxin reductases are characterized by broad substrate specificity and by easily accessible redox centers.[2] In mammalian TrxR, this redox center consists of a cysteine−selenocysteine redox pair that approaches the N-terminal active site of the other subunit for electron transfer.[3] Notably, the active site selenolate group, after reduction, manifests a large propensity to react with "soft" metal ions, making TrxR a likely pharmacological target for a vast array of metallodrugs. This is most likely the reason that various gold(I) and platinum(II) compounds were earlier reported to be potent inhibitors of mammalian thioredoxin reductase.[4−7]

Two main forms of TrxR exist in mammalians: a cytosolic (TrxR1)[8] and a mitochondrial one (TrxR2).[9] The exact physiological role of these two forms is not yet known. Their main function is the reduction of the 12 kDa disulfide protein thioredoxin (Trx) to the corresponding dithiol species.[2,10] The multitude of crucial biological functions performed by the thioredoxin/thioredoxin reductase system makes it an attractive "druggable" target.[11,12] Recent studies suggested that the development of new TrxR inhibitors might be of interest not only for cancer chemotherapeutics but also for the treatment of a variety of diseases such as rheumatoid arthritis, Sjögren's syndrome, and AIDS.[13] Overall, these observations prompted us to ascertain whether selected ruthenium(III) compounds might act as TrxR inhibitors, owing to the appreciable "soft" character of the ruthenium center. To the best of our knowledge, this specific subject had not been addressed.

The field of metallodrugs is a rapidly expanding one, in particular in relation to the search of new antitumor drugs. After the clinical success of cisplatin, much attention was focused on various non-platinum complexes as a rational alternative to cytotoxic platinum drugs.[14] In particular, ruthenium(III) complexes turned out to be very promising for their outstanding antitumor properties associated with a favorable toxicity profile.[15] Notably, two ruthenium(III) compounds, imidazolium *trans*-[tetrachloro(DMSO)(imidazole)ruthenate(III)] (NAMI-A)[16−18] and indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III)] (KP-1019),[19] are currently undergoing clinical trials as anticancer agents (see Chart 1 for structural formulas). In addition, a number of ruthenium(II)−arene compounds were recently shown to possess very encouraging cytotoxic and antitumor properties in preclinical models[20,21] and are now under

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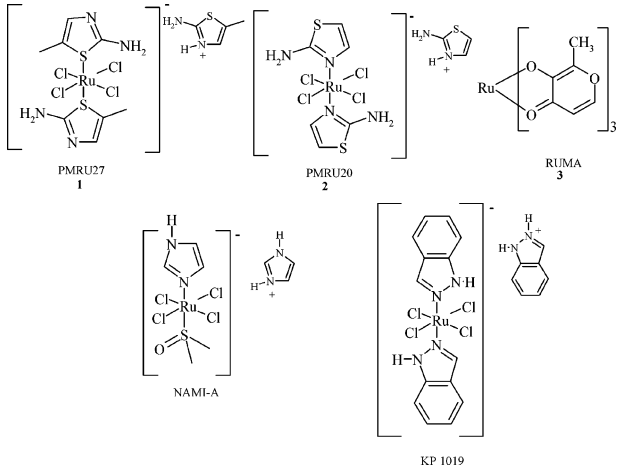
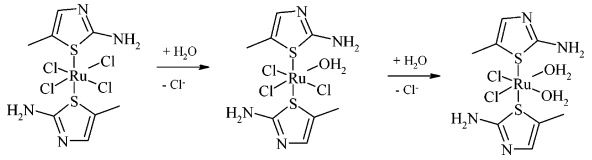
[a] Abbreviations: ICP OES, inductively coupled plasma optical emission spectroscopy; Trx, thioredoxin; TrxR, thioredoxin reductase; TrxR1, cytosolic thioredoxin reductase; TrxR2, mitochondrial thioredoxin reductase.

10.1021/jm0708578 CCC: $37.00
Published on Web 11/02/2007

Chart 1. Schematic Drawing of the Ruthenium Complexes Reported in This Study



Scheme 1. Schematic Represenation of the Proposed Hydrolysis for Complex **1**



active investigation. Yet the effective molecular mechanisms of action and the final targets for anticancer ruthenium compounds have not been elucidated. However, it seems very unlikely that these compounds may target DNA directly as is the case for classical Pt(II) complexes. Alternative hypotheses involving a number of protein targets seem to be more realistic.[22,23]

In the search for novel ruthenium(III) complexes with improved pharmacological profiles, we recently directed our attention to the development of "Keppler type" compounds bearing substituted thiazole ligands.[24,25] Recent studies have firmly established that the chemical and pharmacological properties of the "tetrachlororuthenate core" may be finely tuned through an accurate selection of the axial ligands.[26] Specifically, we report here on a new "Keppler type" compound based on the 2-amino-5-methylthiazole ligand *trans*-[bis(2-amino-5-methylthiazole)tetrachlororuthenate(III)] **1** (PMRU27). Compound **1** was synthesized according to standard procedures with slight modifications, as reported in the SIA. Despite several efforts, we were unable to obtain, for **1**, single crystals suitable for X-ray diffraction analysis. This led us to analyze its microcrystals through powder diffraction methods to determine the most probable structure consisting of a planar tetrachlororuthenate moiety axially coordinated to the sulfurs of two (2-amino-5-methylthiazole) ligands (see Supporting Information).

After completing the structural characterization of **1**, we analyzed its solution behavior through UV−vis absorption spectroscopy (see Supporting Information for details). The UV−vis spectrum of **1** dissolved in aqueous solutions reveals progressive spectral changes over several hours at room temperature (Figure 4S in the Supporting Information). The resulting spectral patterns are fully consistent with those previously reported for 2-aminothiazolium [*trans*-tetrachlorobis(2-aminothiazole)ruthenate(III)][25] (see **2** (PMRU20) in Chart 1) and thus assigned to aquation processes, as described in Scheme 1 and better discussed in Supporting Information. The main electrochemical properties of **1** were investigated as well and found to be in line with those of parent ruthenium(III) complexes as described in Supporting Information.

Afterward, a number of experiments were done to analyze the interactions of **1** with some selected proteins such as human serum albumin, hen egg white lysozyme, bovine erythrocyte ubiquitin, and horse heart cytochrome *c*, mostly relying on ultrafiltration accompanied by spectrophotometric and ICP OES determinations. Overall, the obtained results are suggestive of extensive ruthenium binding to these proteins after occurrence of the first aquation step (in all cases protein-bound ruthenium was found to be ≥70%). Details of these studies are provided in Supporting Information.

In view of its high propensity to react with proteins and form adducts, we decided to investigate whether **1** might behave as an effective inhibitor of mammalian TrxR. The ruthenium(III) center, although less "soft" than the platinum(II) or gold(I) centers, might still manifest an appreciable affinity for the free selenol group of the TrxR active site; moreover, alternative interaction modes of this ruthenium(III) center with other protein side chains might occur as well.[27]

The inhibitory effects of **1** toward cytosolic or mitochondrial forms of rat TrxR were measured according to established procedures (see Supporting Information). For comparison, analogous measurements were carried out on three other ruthenium(III) complexes: **2**, tris-maltolate ruthenium(III) **3** (RUMA), and NAMI A. The four ruthenium compounds were tested at increasing concentrations within the 1−100 μM range. Figure 1 shows the effects of all compounds on cytosolic (TrxR1) and mitochondrial (TrxR2) rat thioredoxin reductase activities. Remarkably, the cytosolic isoform is greatly inhibited, although to different extents, by these ruthenium compounds while the mitochondrial isoform is scarcely sensitive even at the highest tested concentrations. By comparison of the IC_{50} values, **2** appears to be the most effective inhibitor, exhibiting an IC_{50} of 8.6 μM. Similarly, **1** inhibits the enzyme, showing an IC_{50} of 23.8 μM. NAMI A causes a less effective inhibition (IC_{50} = 49.5 μM), while **3** is scarcely effective ($IC_{50} \approx 100$ μM). The inhibitory effect of increasing concentrations of sodium arsenite on TrxR1 and TrxR2 is reported as a positive control. The thiazolidine ligands of **2** (2-aminothiazole) and **1** (2-amino, 5-methylthiazole) were also tested and found to be completely ineffective, even at 200 μM.

To the best of our knowledge, our study represents the first report of relevant TrxR inhibition for ruthenium compounds. Because thioredoxin reductase plays important roles in the overall intracellular redox metabolism and has been proposed to be an attracting target for new anticancer compounds, we believe that the here-described TrxR1 inhibition might be of relevance for the mechanism of ruthenium-based anticancer agents. Moreover, because the thioredoxin/thioredoxin reductase system is implied in a few additional pathophysiological processes, other medical uses different from cancer treatment might be proposed and tested for ruthenium metallodrugs.

A final comment is due to the large difference in sensitivity to ruthenium detected between the two TrxR forms. The behavior of these ruthenium complexes toward the cytosolic and mitochondrial isoforms of TrxR is reminiscent of that found for calcium ions.[28] In fact, while TrxR1 is extremely sensitive to Ca^{2+} inhibition, these ions exert only a weak inhibitory action on the mitochondrial isoform.[28] This difference opens interesting perspectives taking into account the cellular signaling mediated by calcium and hydrogen peroxide because, according to Gitler et al.,[29] the intracellular calcium increase induced by growth factors resulted in a significant conversion of reduced Trx to

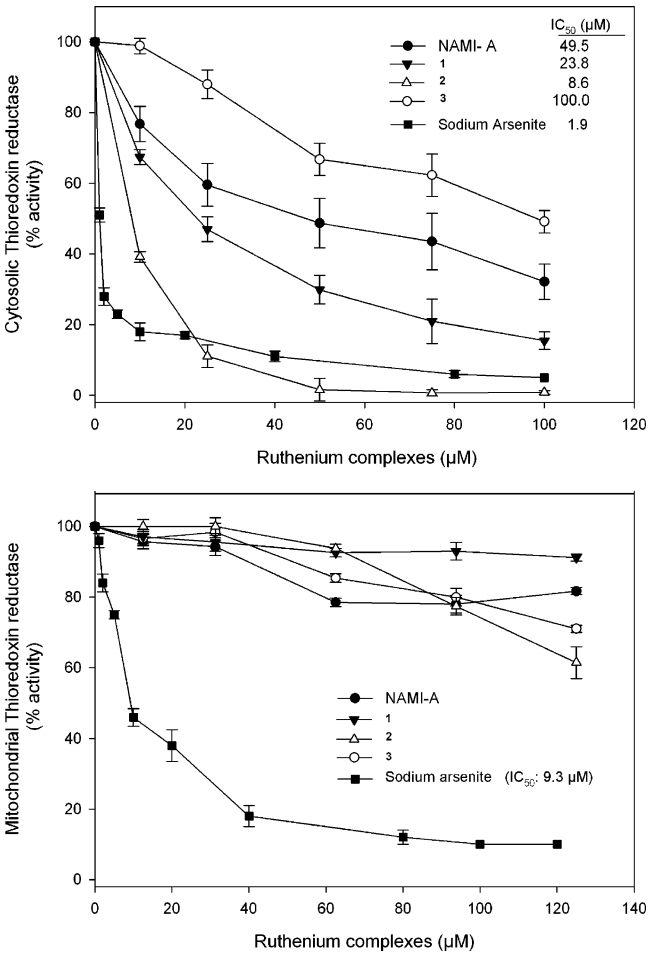


Figure 1. Effect of ruthenium(III) complexes on cytosolic (TrxR1) and mitochondrial (TrxR2) thioredoxin reductases. All the reported values are the mean ± SD of not less than four measurements. The effect of sodium arsenite is reported as a positive control.

its oxidized form. The different response of ruthenium complexes to the two isoforms of TrxR might reside on the different characteristics of the two proteins. According to Sun et al.,[30] the analysis of the protein sequence indicates that the mitochondrial isoform is more basic than the cytosolic enzyme. This condition might explain the lower sensitivity of the mitochondrial TrxR toward aquated ruthenium complexes in comparison with cytosolic TrxR. Furthermore, according to Swiss-Prot/TrEMBL databank, TrxR1 and TrxR2 show theoretical p*I* of 5.95 and 7.98, respectively.

In view of the encouraging biological activity of the here-analyzed ruthenium compounds, further testing is now required to ascertain whether other "Keppler type" ruthenium(III) compounds (or even different kinds of ruthenium(III) compounds) might manifest similar or superior TrxR inhibitory effects. Comparative studies with ruthenium(II) compounds are also warranted.

Acknowledgment. The consortium C.I.R.C.M.S.B, MIUR, and Ente Cassa di Risparmio di Firenze are gratefully acknowledged for financial support. Dr. Angela Casini is grateful to AIRC for providing her with a grant. Thanks are also due to Dr. Angela Altomare (Institute of Crystallography, CNR, Bari) for collecting powder X-ray spectra.

Supporting Information Available: Experimental section that includes information on the synthesis and chemical data for the ruthenium complexes, powder X-ray diffraction studies, electrochemical studies, spectrophotometric data, ICP OES interaction studies with model proteins, and enzyme activity tests. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0708578