Journal of Organometallic Chemistry 783 (2015) 40–45

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Tuning the *in vitro* cell cytotoxicity of dinuclear arene ruthenium trithiolato complexes: Influence of the arene ligand

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ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form
27 January 2015
Accepted 9 February 2015
Available online 18 February 2015

Keywords:
Arene ruthenium compounds
Dinuclear complexes
Thiolato complexes
Anti-cancer compounds

ABSTRACT

A new series of cationic dinuclear arene ruthenium complexes bridged by three thiophenolato ligands, $[(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-SR})_3]^+$ with arene = indane, R = met: **1** (met = 4-methylphenyl); R = mco: **4** (mco = 4-methylcoumarin-7-yl); arene = biphenyl, R = met: **2**; R = mco: **5**; arene = 1,2,3,4-tetrahydronaphthalene, R = met: **3**; R = mco: **6**, have been prepared from the reaction of the neutral precursor $[(\eta^6\text{-arene})Ru(\mu_2\text{-Cl})Cl]_2$ and the corresponding thiophenol RSH. All cationic complexes have been isolated as chloride salts and fully characterized by spectroscopic and analytical methods. The molecular structure of **1**, solved by X-ray structure analysis of a single crystal of the chloride salt, shows the two ruthenium atoms adopting a pseudo-octahedral geometry without metal–metal bond in accordance with the noble gas rule. All complexes are stable in H_2O at 37 °C, but only **1** remains soluble in a 100 mM aqueous NaCl solution, while significant percentages (30–60 %) of **2**–**6** precipitate as chloride salts under these conditions. The 4-methylphenylthiolato complexes (R = met) are highly cytotoxic towards human ovarian cancer cells, the IC_{50} values being in the sub-micromolar range, while the 4-methylcoumarin-7-yl thiolato complexes (R = mco) are only slightly cytotoxic. Complexes **1** and **3** show the highest *in vitro* anticancer activity with IC_{50} values inferior to 0.06 μM for the A2780 cell line. The results demonstrate that the arene ligand is an important parameter that should be more systematically evaluated when designing new half-sandwich organometallic complexes.

Introduction

For more than four decades, *cis*-diamminedichloroplatinum(II) (cisplatin) has been one of the most frequently used anticancer drugs in the chemotherapy of cancer [1]. However, its use is limited by its high systemic toxicity [2] and by the fact that many tumors are either intrinsically resistant or acquire resistance during platinotherapy [3]. Ruthenium-based anticancer compounds have become promising alternatives to platinum-based drugs, ever since the compounds $[H_2im]$*trans*-$[RuCl_4(DMSO)(Him)]$ (NAMI-A, Him = 1*H*-imidazole) and $[H_2ind]$*trans*-$[RuCl_4(Hind)_2]$ (KP1019, Hind = 1*H*-indazole) went into clinical trials; the sodium analogue Na-*trans*-$[RuCl_4(Hind)_2]$ (NKP1339) is now on the edge of clinical application [4]. In general, ruthenium compounds show lower systemic toxicity and higher activity to cancer cell lines. In particular, it has been reported that ruthenium-based drugs are likely to show different modes of action as compared to platinum-based drugs, mainly characterized by interactions with proteins, in particular albumin and transferrin, regulatory enzymes within the cell membrane and DNA inside the cell nucleus [5]. This diversity of action of ruthenium anticancer drugs is also likely to enhance their anticancer activity and to reduce the probability of developing tumor resistance, which is one of the most important limitations of platinum-based drugs.

Organoruthenium complexes of the type $[(\eta^6\text{-arene})Ru(XY)Z]^+$ (where XY is a chelating ligand and Z is a monoanionic ligand) are highly cytotoxic and they also reduce tumor growth *in vivo*. Generally the hydrolysis of the Ru-Z bond, which depends on the nature of the Z ligand, is considered as the first and most important step for the biological activation of this class of compounds, the displacement of this group by rapid hydrolysis makes a coordination site for the bio-target available [6]. However, also the arene

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http://dx.doi.org/10.1016/j.jorganchem.2015.02.010
0022-328X/

ligand plays an important role for this class of compounds, as it stabilizes the metal in its lower oxidation state and provides a hydrophobic face for the passive transport inside cells [7]. Among all the mononuclear arene Ru(II) complexes with anticancer activity known, the RAPTA series, $[(\eta^6\text{-arene})RuCl_2(pta)]$ (pta = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane) displays selective activity on metastatic tumors *in vivo* [8]. The major intracellular target of this class of complexes seems to be proteins [9] and enzymes [10], the hydrolysis of the Ru–Cl bond giving rise to the active targeting species [11]. Furthermore, the possibility of incorporating arene Ru(II) complexes into multinuclear systems including metallacycles [12], metalla-cages [13] and dendrimers [14] has been investigated with the aim of increasing their cytotoxic activity.

We previously reported several series of dinuclear *p*-cymene ruthenium complexes containing three thiolato bridges, $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru_2(\mu_2\text{-}SR)_3]^+$ and $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru_2(\mu_2\text{-}SR^1)(\mu_2\text{-}SR^2)_2]^+$, R and R^1 being aromatic substituents, R^2 being an aliphatic substituent, complexes which turned out to be the most cytotoxic ruthenium compounds reported so far, the IC_{50} values towards the human ovarian cancer cells A2780 and the cisplatin-resistant mutant A2780cisR being in the nanomolar range [15,16]. We observed that these complexes efficiently catalyze the oxidation of cysteine to cystine and of glutathione (GSH) to its oxidized form GSSG, which may partially explain their high cytotoxic activity [17]. A systematic study of the trithiophenolato complexes suggested the cytotoxicity to be influenced by the lipophilicity and the Hammett constants of the corresponding thiol; however, a direct correlation between cytotoxicity, glutathione oxidation activity and redox potentials could not be established [18].

The previous work focused mainly on the impact of the thiolato ligands on the cytotoxicity, with *p*-cymene being systematically used as arene ligand at the ruthenium center. A comparison of the biological activity of dinuclear arene ruthenium trithiolato complexes bearing benzene, hexamethylbenzene or *p*-cymene as arene ligands showed that the arene ligand has indeed an effect on the cytotoxicity; however, the observed IC_{50} values could not be correlated to the lipophilicity or the size of the arene ligands [19]. Herein, we report the synthesis, characterization, molecular structure and the *in vitro* anticancer activity of a new series of six dinuclear arene ruthenium trithiolato complexes with three different arene ligands and two selected thiolato bridges.

Results and discussion

Synthesis and characterization

The arene ruthenium chloro precursors $[(\eta^6\text{-arene})Ru(\mu_2\text{-}Cl)Cl]_2$ (arene = indane, biphenyl or 1,2,3,4-tetrahydronaphthalene) react with an excess of 4-methylthiophenol (metSH) or 7-mercapto-4-methylcoumarin (mcoSH) in refluxing ethanol to afford the new complexes $[(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-}SR)_3]^+$ (**1**–**6**), which are isolated as the chloride salts, air-stable orange to red solids, soluble in ethanol, acetonitrile or dichloromethane, see Fig. 1.

In the ^1H NMR spectra (CD_3CN, 25 °C), complexes **1**–**3** give rise to three signals for the three equivalent *para*-methylthiophenolato ligands: two doublets between 7.0 and 7.8 ppm for the four aromatic protons and a singlet at about 2.4 ppm for the methyl protons. The ^1H NMR spectra of complexes **4**–**6** in $CDCl_3$ exhibit five signals for the three equivalent 4-methylcoumarin-7-thiolato ligands: two doublets at about 8.3 and 7.8 ppm for the C-5 and C-6 protons, respectively, a singlet at about 7.7 ppm for the C-8 proton and two singlets at 6.4 and 2.5 ppm for the C-3 proton and the methyl protons in C-4, respectively. The coordination of the arene ligand to the ruthenium center in **1**–**6** causes a characteristic high-field shift for the arene protons in the coordinated part,

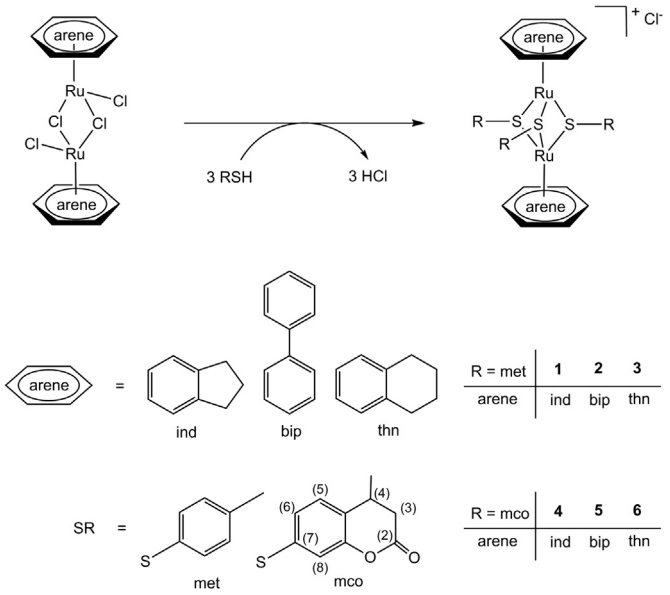

Fig. 1. Reaction scheme showing the arene and thiolato ligands used in this work (including the atom numbering scheme for the coumarin system).

characteristic for organometallic half-sandwich complexes [23,24]. On the other hand, the proton resonances of the arene moieties that are not coordinated experience only marginal chemical shift changes as compared to the arene ruthenium chloro precursors.

Single-crystal X-ray structure analysis

The molecular structure of **1** was established by X-ray diffraction analysis of the chloride salt. A single crystal of **[1]**Cl · $CHCl_3$ was obtained by recrystallization from a chloroform/diethyl ether mixture. The ORTEP drawing with the atom labeling scheme for cation **1** shown in Fig. 2 contains a trigonal–bipyramidal Ru_2S_3 moiety, in which each ruthenium atom adopts a pseudo-tetrahedral geometry, owing to the presence of three sulfur atoms and the indane ligand that formally occupies three coordination sites.

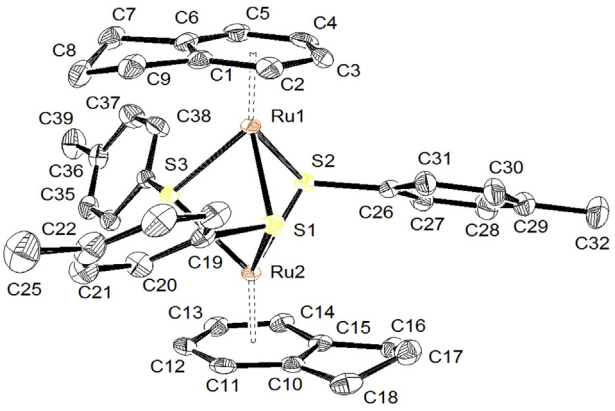

Fig. 2. ORTEP drawing of cation **1**, the thermal ellipsoids being drawn at 50% probability level and hydrogen atoms as well as the anion and the solvent molecule being omitted for clarity. Selected bond lengths (Å) and bond angles (°): Ru(1)–Ru(2) 3.3544(4), Ru(1)–S(1) 2.4201(16), Ru(1)–S(2) 2.3786(16), Ru(1)–S(3) 2.4164(14), Ru(2)–S(1) 2.3929(15), Ru(2)–S(2) 2.4086(16), Ru(2)–S(3) 2.4073(15); Ru(1)–S(1)–Ru(2) 88.36(5), Ru(1)–S(2)–Ru(2) 88.96(3), Ru(1)–S(3)–Ru(2) 88.12(4).