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From Pyrone to Thiopyrone Ligands−Rendering Maltol-Derived Ruthenium(II)−Arene Complexes That Are Anticancer Active in Vitro

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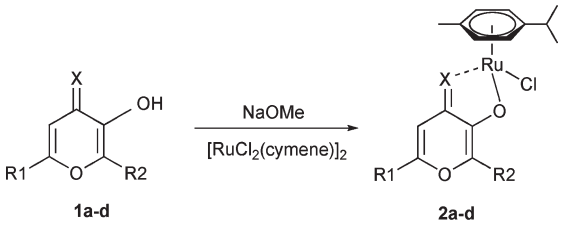
Summary: Ru(II)−arene complexes with pyrone-derived ligands are rendered active against cancer cells by replacement of the coordinated O,O donor with an S,O donor. The different stabilities of these systems may explain the observed influence of the donor atoms on the anticancer activity in vitro.

Metal complexes are playing an important role in the treatment of cancer, and many promising compounds have been developed in recent years.[1−4] Ruthenium complexes have been shown to be among the most promising candidates for new metal-based anticancer drugs. Two of them, KP1019 and NAMI-A, are currently undergoing clinical trials.[2,5] Their low general toxicity might be explained by their modes of action, including protein binding and activation by reduction.[5−7]

More recently, bioorganometallic chemistry has emerged as a new source of anticancer metallodrugs, with titanocene dichloride being the prototype agent of this compound class.[4,8,9] Furthermore, organometallic Ru(II) compounds that are stabilized in their +2 oxidation state by coordination of an arene ligand have been investigated for their anticancer properties. These piano-stool complexes have been pioneered by the Dyson and Sadler groups,[10,11] who developed compounds with pta (1,3,5-triaza-7-phoshatricyclo[3.3.1.1]-decane) and en (ethylenediamine) ligands, respectively.[10] For the [(η^6-arene)Ru^{II}(X)(Y)] complexes, DNA base selectivity strongly depends on the character of the chelating ligand Y− exchange of the neutral ethylenediamine by anionic acetylacetonate shifts the affinity from guanine to adenine.[12] In addition to en and pta complexes, maltol-derived mono- and polynuclear ruthenium and osmium complexes have been developed.[13−15] The linking of two pyridone moieties opened up new possibilities for tuning the in vitro anticancer activity and lipophilicity, and compounds with interduplex cross-linking capacity were obtained.[14,16−18] In the case of the mononuclear Ru(II) complexes, an increase in cytotoxic activity was achieved by derivatization of the pyrone ring with lipophilic aromatic substituents.[13]

Scheme 1. Synthesis of the Complexes 2a−d



a R1 = H, R2 = CH_3, X = O
b R1 = H, R2 = CH_3, X = S
c R1 = CH_3, R2 = H, X = O
d R1 = CH_3, R2 = H, X = S

In order to study the Ru−ligand interaction and its effect on the in vitro anticancer activity, Ru(II)−cymene complexes (Scheme 1) with pyrones and their corresponding, more lipophilic thiopyrones as chelating agents were prepared.[15,19] Such (thio)pyrone systems have already found application in

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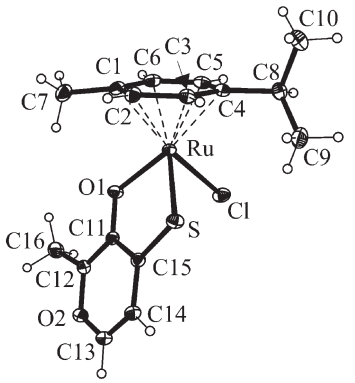

Figure 1. ORTEP plot of the molecular structure of **2b**.

medicinal chemistry.[20,21] The Ru(II)−cymene complexes were obtained in good yields (64−85%) by deprotonation of the ligand with sodium methoxide and reaction with bis[dichlorido-(η^6-*p*-cymene)ruthenium(II)]. The reaction was performed with a slight excess of ligand to facilitate the purification. All complexes have been fully characterized by 1D and 2D NMR spectroscopy, mass spectrometry, and elemental analysis.

Single crystals of **2b** were obtained from ethyl acetate, and the molecular structure was determined by X-ray diffraction analysis. The ruthenium center was found to adopt a pianostool configuration (Figure 1 and the Supporting Information). Due to the larger sulfur atom in **2b**, as compared to the oxygen in maltol-derived ligands, the Ru−S bond (2.3730(3) Å) is significantly longer than the Ru−O bond (2.0808(10) Å). This leads to a strong distortion of the five-membered chelate ring of the complex. The length of the Ru−Cl bond in **2b** (2.4331(4) Å) is comparable to the corresponding bond lengths of pyrone and pyridone Ru(II)−arene complexes.[14,15]

The antiproliferative activities of **2a**−**d** were investigated in the human tumor cell lines SW480 (colon carcinoma) and CH1 (ovarian carcinoma) by using the colorimetric MTT assay (Figure 2). The IC_{50} values are presented in Table 1. As reported earlier,[14] the maltol complex **2a** shows limited cytotoxic activity, and the allomaltol derivative **2c** has IC_{50} values $> 200\ \mu$M. In contrast, the thiopyrone complexes **2b**,**d** are at least by an order of magnitude (in IC_{50}) more active than their pyrone analogues. For complexes **2b**,**d**, an inverted sensitivity of SW480 cells and CH1 cells was observed, which is in contrast with the case for a broad spectrum of other compounds but parallels that observed with Ru(II)−arene complexes containing an 8-quinolinolato ligand.[22] The substitution pattern influences the activity, as inferred from the 2.7−3.9 times higher activity of **2b** as compared to **2d**. These compounds are less cytotoxic than other ruthenium complexes[23] but are still in a reasonable range of activity in vitro in comparison to other Ru−arene species.[22] However, the in vitro effects are only a first indication for potential activity, which needs to be verified in vivo.

The in vitro inactivity of the maltolato complexes has been attributed to the formation of dimers in aqueous solution.[13]

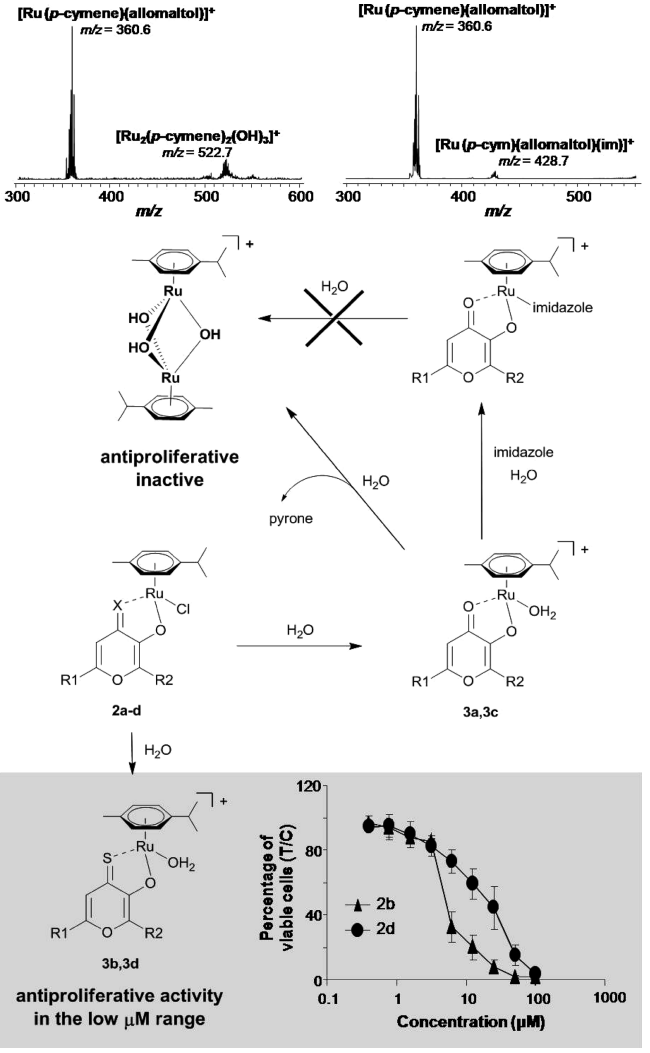

Figure 2. Hydrolysis of Ru−(thio)pyronato complexes (X = O, S), yielding in the case of the pyrone ligands the dimeric $[Ru_2(cym)_2(OH)_3]^+$ species (as demonstrated by ESI-MS; top left). When the aqua complexes **3a**,**c** were reacted with imidazole, no dimerization was observed (top right), as was the case for the thiopyrone compounds **3b**,**d**, which show significant cytotoxic activity in human cancer cell lines (bottom).

Accordingly, hydrolysis and stability in water were investigated by NMR spectroscopy in D_2O or 10% DMSO-d_6/90% D_2O solutions for the maltol and thiomaltol compounds, respectively. The complexes **2a**−**d** hydrolyze within seconds to charged complexes by exchange of the chlorido ligand with a water molecule (Figure 2). No coordination of DMSO-d_6 was observed, and identical NMR spectra were obtained by activation of **2a**,**c** with an equimolar amount of $AgNO_3$, which confirms the obtained results. Similarly to the case of **2a**,[13] the formation of a dimeric species was observed for **2c** (Figure 2, top left), as demonstrated by ESI-MS with a sample of 25 μM; this concentration was chosen in order to obtain a mass spectrum containing both the intact complex and the dimeric species. The amount of side product formed depends on the concentration, time, and pH value of the solution, and its formation can be prevented by addition of an equimolar amount of imidazole, as also demonstrated by means of ESI-MS (Figure 2, top right).

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Table 1. Antiproliferative Activity, ΔG of Hydrolysis, and Bond Energies (kcal/mol) of 2a−d (2′a−d)

	IC_{50} (μM)			
	2a	**2b**	**2c**	**2d**
CH1	> 100	13 ± 4	239 ± 22	35 ± 8
SW480	> 100	5.1 ± 0.5	359 ± 119	20 ± 7
	2′a	**2′b**	**2′c**	**2′d**
ΔG_{hydr}	4.2	3.3	4.9	3.6
E(Ru−Cl)	10.6	7.1	11.1	7.9
$E(Ru-O/S)_{O/S=C}$	26.8	34.6	26.4	34.4
$E(Ru-O)_{O-C}$	36.9	35.7	36.6	36.2

Hydrolysis of **2b**,**d** in 10% DMSO-d_6/D_2O results in the rapid formation of aqua species, which are stable for more than 24 h; in contrast to the case for the maltolato complexes, no dimers have been observed. To explain these observations, theoretical DFT calculations (B3LYP, Gaussian 03 program package;[24] see the Supporting Information for computational details) of the metal−ligand bond energies have been performed. They reveal that the $Ru-S_{S=C}$ bond energies in the model complexes **2′b**,**d** (bearing benzene instead of cymene in **2b**,**d**) are higher by 7.8−8.0 kcal/mol than the $Ru-O_{O=C}$ bond energies in **2′a**,**c**, respectively. In contrast, the $Ru-O_{O-C}$ bond energies in thiopyrone complexes are lower than those in pyrone species, but only by 0.4−1.2 kcal/mol. The stronger binding of thiopyrones to Ru as compared to pyrones explains why thiopyrone complexes are stable in aqueous solution while pyrone complexes undergo decomposition with release of the ligands **1a**,**c**.

Theoretical calculations of **2′a−d** and **3′a−d** show that ΔG values for the substitution of chloride with H_2O are only slightly positive (3.3−4.9 kcal/mol). Taking into account the large excess of water, complexes **3a−d** are the predominant species in aqueous solutions. Lower Ru−Cl bond energies in **2′b**,**d** as compared to **2′a**,**c**, respectively, correlate with ΔG values of hydrolysis (Table 1).

In summary, the modification of the first ligand sphere and therewith of the stability of the complexes influences significantly the in vitro anticancer activity. Pyrone and thiopyrone compounds behave quite differently in water (dimer formation). These features have important implications for the mode of action of the compounds and result in considerably active thiopyrone vs minimally active pyrone complexes. Furthermore, complexes **2b**,**d** are slightly more active in SW480 cells, though CH1 cells are in our experience more chemosensitive to the vast majority of metal-based and other tumor-inhibiting compounds tested so far.

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Supporting Information Available: Text, tables, figures, and a CIF file giving the general procedure for the synthesis of the complexes, characterization, elemental analysis data, X-ray diffraction data, and computational details. This material is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data for this paper are also available from the Cambridge Crystallographic Database (CCDC 720741).

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